

HELGE OLSEN

FLAVOBACTERIUM MENINGOSEPTICUM

A bacteriological
epidemiological and clinical study



I kommission hos
ANDELSBOGTRYKKERIET I ODENSE
1969

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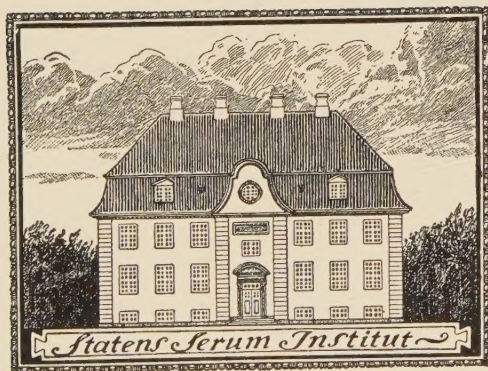
Odense universitet, den 15. december 1967.

HELGE OLSEN

FLAVOBACTERIUM MENINGOSEPTICUM

A bacteriological,
epidemiological and clinical study

Odense County and City Hospital
and
Statens Seruminstitut



I kommission hos
ANDELBOGTRYKKERIET I ODENSE
1969

Denne afhandling er af det natur- og lægevidenskabelige fagråd ved Odense Universitet
antaget til offentligt at forsvares for den medicinske doktorgrad.

Odense Universitet, den 15. januar 1969.

Franz Bierring

h. a. dec.

Til mine forældre

Earlier publications

1. Olsen, H., W. C. Frederiksen & K. E. Siboni: *Flavobacterium meningosepticum* in 8 non-fatal cases of postoperative bacteraemia. *Lancet* 1965, 1: 1294-1296.
2. Olsen, H.: The importance of temperature for the growth of *Flavobacterium meningosepticum*. *Acta path. microbiol. scand.* 1966, 67: 291-302.
3. Olsen, H.: A clinical analysis of 10 cases of postoperative infection with *Flavobacterium meningosepticum*. *Dan. med. Bull.* 1967, 14: 1-5.
4. Olsen, H.: An epidemiological study of hospital infection with *Flavobacterium meningosepticum*. *Dan. med. Bull.* 1967, 14: 6-9.
5. Olsen, H.: An in vitro study of the antibiotic sensitivity of *Flavobacterium meningosepticum*. *Acta path. microbiol. scand.* 1967, 70: 601-612.
6. Olsen, H.: The sensitivity of *Flavobacterium meningosepticum* to antibiotics at different temperatures. *Acta path. microbiol. scand.* 1967, 71: 592-598.
7. Olsen, H.: *Flavobacterium meningosepticum* isolated from outside hospital surroundings and during routine examination of patient specimens. *Acta path. microbiol. scand.* 1969, 75: 313-322.

The monograph contains a number of investigations which have not been published previously.



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Preface

This study started in the spring of 1964 during my appointment as senior registrar to the Department of Thoracic Surgery, Odense County and City Hospital, and was completed during my appointment as registrar in Statens Seruminstitut, Regional Laboratory in Odense, where all the bacteriological investigations were carried out.

My thanks are due to the Head of the Department of Thoracic Surgery, H. Rahbek Sørensen, M.D., for the ideal working conditions, and for his encouragement and inspiration during the early stages of the study.

K. E. Siboni, M.D., Head of Statens Seruminstitut, Regional Laboratory in Odense, introduced me to bacteriological technique and biological thinking in the course of frequent talks and discussions. He followed the investigation with never-failing interest and offered valuable criticism. Without his continued support it would not have been possible to complete the study.

The personale of many departments of Odense County and City Hospital have willingly given me their help. In particular my thanks are due to Aase Hjorth, M.D. and S. Jørgensen, M.D., Heads of the Anaesthetic Department, and to the nursing staff of the Anaesthetic Department and the Department of Thoracic Surgery. A number of oxygen analysis were performed in the Laboratory for Clinical Physiology, whose Chief, J. Fabricius, M.D., I thank for this, and for our productive talks.

Laboratory assistants Miss Dina Jørgensen and Mrs. Lone Krogh have given me valuable and meticulous assistance in the laboratory work.

A number of members of the staff of Statens Seruminstitut have followed this investigation with interest. I am indebted to H. Lautrop, M.D. for a number of instructive talks, to S. Olesen Larsen, M. A. for his assistance with statistical problems, to W. C. Frederiksen, M.D., for carrying out serological investigations, and to Miss Aase Langhorn, B. Sc., for preparing the substrates.

The manuscript has been translated into English by Dr. Jill Gordon Klee, and typed by Miss Agnete Thorborg.

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Copenhagen, March, 1969

Helge Olsen

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Introduction

This monograph contains a report of a hospital epidemic caused by *Flavobacterium meningosepticum* in which the micro-organism was isolated post-operatively from the blood of adult patients. In addition the biochemical properties of *Flavobacterium meningosepticum* are described, together with its sensitivity to antibiotics and growth at various temperatures. In conclusion, a description is given of the investigations which have been carried out with the aim of clarifying the epidemiological conditions and the occurrence of the micro-organism in nature.

Flavobacterium meningosepticum has been isolated from cerebrospinal fluid and blood of premature infants with severe meningitis occurring endemically in hospitals. This was first reported by Vandepitte et al. (1958) but subsequently a number of similar endemics have been described (Brody et al. 1958; Buttiaux & Vandepitte 1960; Cabrera & Davis 1961; George et al. 1961; Seligmann et al. 1963; Sugathadasa & Arseculeratne 1963; Vandepitte et al. 1965; Watson et al. 1966; Plotkin & McKittrick 1966). A total of 63 cases of meningitis in premature infants caused by *Flavobacterium meningosepticum* have been described. The majority have occurred in U.S.A., but cases have also been reported from Ceylon, Congo, Israel and South Africa. The disease would thus seem to be localized especially to areas with a hot climate.

Flavobacterium meningosepticum is a gram-negative, non-motile rod, which was first characterized as a species by the American bacteriologist Elizabeth O. King (1959). She also described six serologically different types which she named A-F (King 1965).

As early as in 1944 Shulman & Johnson described a case of meningitis in a premature infant which was undoubtedly due to *Flavobacterium meningosepticum*. Biochemically the strain isolated deviated from *Flavobacterium meningosepticum* only in that it produced acid from salicin.

Apart from the cases described by the present author, only one certain case of *Flavobacterium meningosepticum* infection in an adult has been reported; a 69-year-old woman with meningitis (King 1959). In a second patient, with bacteraemia following tooth extraction, the causative organism may have been *Flavobacterium meningosepticum*, but the bacteriological investigations reported were inadequate to determine this with certainty (Pinter & Ivanyi 1965).

Human infections with Flavobacteria which definitely differ from *Flavobacterium meningosepticum* have been described. There is a report of a case of endocarditis

(Schiff 1961) and Berry et al. (1963) describe four cases of post-operative bacteraemia in which the source of infection was a contaminated heart-lung machine.

In Bergey's Manual of Determinative Bacteriology (1957) the genus *Flavobacterium* includes 26 species, none of which is identical to *Flavobacterium meningosepticum*. They show considerable biochemical and morphological differences, which would suggest that the group is very inhomogeneous. Desoxyribonucleic acid analyses, in which the results from related strains differ only slightly, have also given widely deviating values in investigations of *Flavobacteria* (Hill 1966).

Factors of importance in the treatment of premature infants with meningitis

All adult patients suffering from *Flavobacterium meningosepticum* infections have recovered without sequelae, but the prognosis in premature infants is poor. Of 53 reliable reports of cases the infection was fatal in 39, whilst only four of the survivors did not have sequelae, the most common of which was hydrocephalus. It is recognized that many other infections have a severe course in premature infants, due to their low resistance. It has been demonstrated that the phagocytic ability of the leucocytes is lowered in premature infants (Gluck & Silverman 1957), whilst the same is true of the production of antibodies (Gluck et al. 1966).

The fact that premature infants have an increased meningeal permeability (Otila 1948) could be expected to potentiate antibiotic treatment, as it is to be expected that it will be possible to achieve higher concentrations of antibiotics in the cerebrospinal fluid. Furthermore the serum concentration of antibiotic will be higher in premature infants than in mature infants and adults after the administration of the same dose per kg body weight. This is due to the slower renal excretion in the premature, and has been demonstrated for kanamycin, neomycin, streptomycin, ampicillin, methicillin and oxacillin. By contrast, it does not hold true for polymyxin (Axline & Simon 1964; Axline et al. 1967).

Premature infants with meningitis often have no fever (Groover et al. 1961). This might be thought to be unfavourable for antibiotic therapy, as the meningeal permeability seems to increase with the temperature. It has thus been demonstrated that higher concentrations of penicillin were achieved in the cerebrospinal fluid of patients with cerebral syphilis when they had fever (Dewhurst & Todd 1965). Moreover, there are several observations which indicate that the effect of a number of antibiotics is accentuated when the temperature rises. This has been demonstrated for the action of the sulphonamides on β -haemolytic streptococci (White 1939), for isoniazide, streptomycin and para-aminosalicylic acid on *Mycobacterium tuberculosis* (Marks 1951; Arriagade 1952; Knox et al. 1952) and for chloramphenicol on *Salmonella typhi* (Fassin et al. 1955). The action of chloramphenicol and erythromycin on *Staphylococcus aureus* was found to be increased with temperature by Christoffersen & Hove (1967), whilst in contrast Waterman et al. (1967) found that the effect of chloramphenicol on *Staphylococcus aureus* and *Escherichia coli* fell with increasing temperature. Further these last authors found that the activity of sodium nafcillin on *Staphylococcus aureus* improved with increasing temperature. The effect of penicillin and tetracycline was

independent of the temperature in the range 30°–45°C; it was perhaps slightly better at 30°C than at the higher temperatures.

Drugs other than antibiotics also show increased activity with increasing temperature. This has been demonstrated in rat experiments for-dicoumarol (Richards 1943). In the mouse the convulsive effect of insulin was found to show a marked increase with increasing temperature (Chen et al. 1943). The average rectal temperature in the mice during the experiments ranged from 34.65° to 38.48°C, but within this modest range of temperature the insulin effect was increased by more than 80 times. The phenomenon has also been observed in cold-blooded animals. In frogs it has thus been found that there is an increase of the activity of the digitalis-like compounds cymarin and coumagine with increasing temperature (Chen et al. 1943).

There are a few reports which indicate that the phenomenon described may be of therapeutic importance. Satisfactory results have thus been obtained in the treatment of *Salmonella* infections with a combination of chloramphenicol and fever therapy (Laporte et al. 1950; Berger 1951).

Another disease in which there would seem to be justification for the use of hyperthermic therapy from a theoretical viewpoint is tuberculous meningitis. In this disease, which still has a high mortality rate, hyperthermic therapy would enable advantage to be taken of both an increased activity of the antibiotics and an increased meningeal permeability.

Micro-organisms and temperature

As temperature conditions play an important part in this study, some of the findings regarding the influence of temperature on micro-organisms will now be described.

I. Pathogenicity and temperature

In order that a micro-organism can be pathogenic there is one usual, if not absolute precondition: that the organism is capable of reproduction at the temperature present in the host organism. However, this naturally does not imply that all such micro-organisms are pathogenic.

However, a micro-organism may well be biologically active at temperatures at which it is not able to divide, as the optimum temperatures for others of its functions may differ from that at which the rate of division is optimum. For example in *Streptococcus lactis* and *Streptococcus thermophilus* the optimum temperature for fermentation has been found to be 6° and 10°C, respectively, higher than the optimum temperature for division (Dorn & Rahn 1939). Alford (1960) has demonstrated for a number of strains of *Pseudomonas* and *Achromobacter* that the biochemical properties often have a lower optimum temperature than the ability to divide.

The temperature in a macro-organism is not the same throughout. In man the temperature of the skin is several degrees lower than the rectal temperature, and the same is true of the temperature in the scrotum. The pulmonary temperature in man has been found to be 35.2°–35.6°C (Loewy & Gerhartz 1914). The temperature in the liver in the dog, rabbit and pig has been found to be about 1°C above the rectal temperature (Lefevre 1911). It will therefore be possible for a micro-organism to reproduce in some of the organs in man even if it is incapable of division at 37°C.

The available reports of the growth of bacteria at different temperatures often lack details of the methods employed. One exact method, which has been used only during recent years, is the employment of thermogradients (Oppenheimer & Drost-Hansen 1960; Cannefax 1962; Nakae 1966). As yet, however, only a few strains have been investigated by this means.

The following table (Table 1) which, with the exception of the values for the last bacterium shown in the table, is adapted from Topley & Wilson (1955), shows the optimum growth temperatures for a number of bacteria, together with the range of temperatures at which growth can occur. The bacteria are divided into three groups:

A. Pathogenic bacteria which rapidly succumb outside the human body have optimum at 37°C and a very narrow temperature range for growth.

B. Pathogenic bacteria which are viable outside the human body have optimum growth at 37°C but are capable of growth through a wider range of temperature than group A.

C. Bacteria which are rarely or never pathogenic to man have an optimum growth temperature other than 37°C.

Table 1

	Optimum temperature for growth (°C)	Range of temperature over which growth is possible (°C)
A. Bacteria pathogenic only to man and not viable outside the human organism		
Neisseria gonorrhoeae	37	30-38.5
Neisseria meningitidis	37	30-38
Treponema pallidum	rabbit testicles are sterilized by exposure to 39°C for 5 hours	
B. Bacteria pathogenic to man but viable outside the human organism		
Staphylococcus aureus	37	12-45
Eschericia coli	37	15-45
Vibrio cholera	37	16-42
Salmonella paratyphi	37	15-42
Leptospira icterohaemorrhagiae		25-37
Pseudomonas aeruginosa	30-37	5-42
C. Bacteria rarely or never pathogenic to man		
Serratia marcescens	25-30	
Chromobacterium violaceum	25	
Pseudomonas fluorescens	25	
Bacillus brevis	35	10-45
Bacillus subtilis (Nakae 1966)	39	20-50

Optimum growth temperature and range of temperature at which growth can occur for some bacteria (from Topley & Wilson 1955).

The thermolability of the bacteria in group A is so pronounced that marked inhibition of growth may be expected at temperatures which are easily reached in the human body. In syphilis and gonorrhoea hyperthermia has also been found to have a definite clinical effect, and a number of clinical observations have confirmed the therapeutic effect of increase in temperature in these diseases. Gonorrhoea is normally not associated with fever, but if the disease is complicated by epididymitis the patient develops a high temperature. In such cases the bacteria rapidly disappear from the urethral secretion, and the prognosis is better than in cases without epididymitis (Haxthausen 1949). In complicating arthritis, in which there is also high fever, the synovial fluid is usually sterile (Langenskiöld 1949). Dementia paralytica is very rare in regions in which such febrile diseases as smallpox and malaria are endemic

(Jacobowsky 1965). There are also reports of the beneficial effect of hyperthermia in meningococcal meningitis (Lampert 1948).

The body temperatures which may be reached in man will have little or no influence on the capacity of division of the bacteria named in group B. However, *Leptospira icterohaemorrhagiae*, which causes Weil's disease, is more thermolabile than the other pathogenic bacteria shown in this group. It is also found in Weil's disease that the micro-organism disappears from the blood within a few days of the onset of the rise in temperature characteristic of that disease.

Some of the bacteria in group C show no growth at 37°C; this is the case in *Pseudomonas fluorescens* (Jessen 1965). Among the *Chromobacterium violaceum* there are some strains which do not grow at 37°C, and others which grow at temperatures up to 42°C. The thermoresistant strains are described in Bergey's Manual (1957) as a special species, *Chromobacterium janthium*, and it is presumably such strains which have caused serious high-febrile infections in man (Black et al. 1938). Similar infections have also been described in monkeys (Audebaud et al. 1954).

The maximum temperature for growth of *Serratia marcescens* has varied in different reports. Bergey's Manual (1957) states that there is no growth at 37°C. One strain isolated from contaminated blood grew poorly at 37°C but well at 22°C (Black et al. 1967). Jennison (1935) found good growth at 37°C but no growth at 42°C, whilst Stenderup et al. (1966) found good growth at 38°C in a strain isolated during a hospital endemic in premature infants. During this endemic there were seven cases of bacteraemia, and six of the infants died. Presumably the thermoresistance of this strain, in association with the fact that the patients did not develop fever, has played a part in the serious course of the infections. Infections with *Serratia marcescens* are rare in man, but would seem to be on the increase (Graevinitz 1964). One case has been described from Denmark by Eriksen & Jarnum (1960). As was the case with *Chromobacterium violaceum*, it must be assumed that *Serratia marcescens* infections in man are also due to the more thermoresistant strains.

The difference between the growth of bacteria at different temperatures is employed in bacteriological diagnosis. *Pseudomonas aeruginosa* may be distinguished from other fluorescent species of *Pseudomonas* by its ability to grow at 42°C. It is easier to isolate *Listeria monocytogenes* from faeces when the specimen is incubated at 4°C and polymyxin added (Bojsen-Møller 1962).

II. Mutants which deviate with respect to temperature sensitivity

Mutants which react differently to the influence of temperature as regards growth are known in a number of bacteria, including *Escherichia coli* (Maas & Davis 1952; Cousin 1967), *Proteus vulgaris* (Hamon & Peron 1967), *Salmonella typhimurium* (Parada & Ortega 1967), and *Salmonella senftenberg* (Davidson et al. 1966).

A micro-organism which is unable to reproduce in the human organism because of thermolability may become capable of this by mutation, and perhaps by this means become a pathogen. It is presumably also possible that micro-organisms which be-

cause of temperature sensitivity thrive poorly in regions with hot climates, might become capable of this by mutation. It is therefore to be anticipated that there are strains of bacteria in the tropics which thrive at a higher temperature than the corresponding strains in temperate and arctic regions.

In the present study it is demonstrated that there are strains of *Flavobacterium meningosepticum* whose growth is already inhibited at 37°C, and also more thermo-resistant strains whose growth is not inhibited until the temperature reaches 40°C. The thermolabile strain is of low pathogenicity, and has been demonstrated only in temperate regions, whilst the thermoresistant strain is highly pathogenic and has been demonstrated in regions with a hot climate. These differences in pathogenicity and geographic extension can be correlated on the basis of the considerations mentioned above.

Similar findings have been demonstrated for other micro-organisms. *Azotobacter* strains isolated in Arizona, which has a hot climate, were found to retain more nitrogen at high temperatures than laboratory strains of *Azotobacter* isolated in Vienna and the northern parts of the U.S.A., where the climate is cooler (Greene 1932).

In temperate zones it has been found that production of gas in MacConkey's broth is almost specific to enterobacteria of faecal origin (Wilson et al. 1935; Ferramola 1940). This does not hold true in certain tropical regions. In India it has been demonstrated that 63.3% of the strains which produced gas in this medium were also indole-negative, methyl red-negative, Voges-Proskauer-positive and citrate-positive (Raghavachari & Iyer 1939). In Singapore 13.3% of such strains were found (Boizot 1941). This means that "Klebsiella" strains are more capable of gas production in MacConkey's broth in the tropics than in temperate zones. However, as the strains were identified only by the four properties named, it is not established whether they were the same species in the two geographic areas.

Brock (1967) investigated the photosynthesis in blue-green algae isolated from hot springs with temperatures varying from 33.2° to 71.2°C. The photosynthesis was optimum at the temperature prevalent in the place where the alga was isolated.

Analogous conditions have been observed in a higher organism such as *Drosophila funebris*. Populations from hot regions tolerated heat better and cold less well than populations from cooler regions. Populations from regions with continental climate were able to tolerate both heat and cold (Timoneeff-Ressovsky 1935).

More surprising than the ability of bacteria to adapt to higher temperatures is the isolation of obligate thermophilic bacteria from localities in which the temperature is low. Bartholomew & Rittenberg (1949) found on the sea-bed, where the temperature was below 10°C, bacteria which grew at 60°C but not at 37° and 30°C. There have been corresponding findings in arctic regions (McBee and McBee 1956). One possible explanation of this may be that in the laboratory the environment to which the bacteria are exposed in nature has not been reproduced. ZoBell (1952) has demonstrated that the growth of bacteria isolated from great depths of the sea is promoted

by high hydrostatic pressures, and there are a number of examples of bacteria which will only grow at a particular temperature in the presence of specific nutrient factors. These are usually certain of the amino acids and vitamins of the B group (Precht et al. 1955; Maas & Davis 1952; Pareda & Ortega 1967).

III. Arrhenius' law

In contrast to many other factors of biological importance, the temperature in any micro-organism is always equal in the cell and the macro-environment.

The temperature affects the micro-organism through its influence on the chemical processes. The correlation between temperature and the rate of a chemical reaction has been described by Arrhenius by the formula

$$k = Ae^{-\frac{E}{RT}}$$

where k is the rate when the concentration of the reactants is molar, T is the temperature in degrees Kelvin, and R is the gas constant. The constants A and E represent the frequency factor and the activation energy, respectively (see e. g. Jensen 1959).

If the formula is written as

$$\ln k = \ln A - \frac{E}{RT}$$

it can be seen that there is a linear relation between the logarithm of the reaction rate and the reciprocal value of the absolute temperature.

Enzymes act by reducing the activation energy (E) such that the rate at any given temperature is increased.

As the rate of an enzymatic process is proportional to the amount of enzyme, and as enzymes are destroyed with increasing temperature, deviations from Arrhenius' law are found in the enzymatic processes. The rate will increase with temperature to a maximum (optimum temperature of the enzyme) after which any further increase in temperature will cause a steep fall due to the decreasing amount of enzyme.

Many enzymatic processes are usually involved in any biological system. Every definite function in such a system will have its optimum temperature, close to the temperature which is optimum for that enzyme involved in the process which is most sensitive to temperature.

The rate of division of bacteria at different temperatures corresponds to the dependence of the enzymatic effect on temperature (Fig. 1). The graphs of the rates of division of various bacteria as a function of the temperature differ by having different optimum temperatures, as an expression of the fact that the enzyme systems which are involved in the bacterial division have different optimum temperatures. The sharp fall in rate of division at temperatures above the optimum indicates that an alteration of temperature here causes a far greater change in the rate of division than does a corresponding change in temperature at temperatures below the optimum.

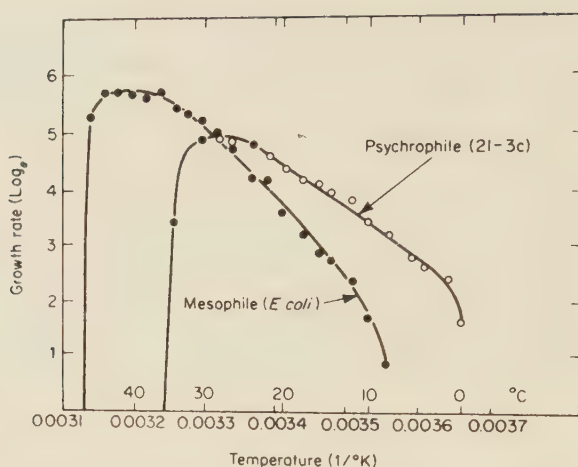


Fig. 1. Relationship between growth rate and temperature for a psychrophile strain and a mesophile plotted in an Arrhenius system (after Ingraham 1962).

For this reason it is easier to determine the maximum growth temperature exactly than to determine the minimum.

At temperatures below the optimum the graphs in Fig. 1 are rectilinear over a certain range of temperature, indicating that the reaction follows Arrhenius' law in this range. It has been demonstrated that a number of widely differing biological processes also follow Arrhenius' law. Of these can be mentioned the heart rate in man (Hoagland & Perkins 1934), the rate at which insects crawl (Crozier 1925) and the frequency of intracellular potentials of the thorax of the mouse (Feigen et al. 1963). Even such a complex process as the frequency of α -rhythm in the human cerebrum has been found to follow this law (Hoagland 1936).

In numerous biological processes it has been demonstrated that there is break in the graphs drawn in an Arrhenius system (Crozier 1925). This is also the case in the rate of division of bacteria; this is apparent from both graphs in Fig. 1 at growth temperatures approaching the minimum, and similar observations have been made by other workers (Johnson et al. 1954; Johnson 1957). Crozier (1925) has explained this break as the result of a new enzyme system becoming predominant when the temperature is changed above a certain limit. Other authors have put forward a different possible explanation for the break (Lavergne & Drost-Hansen 1956; Drost-Hansen 1956; Oppenheimer & Drost-Hansen 1960). These authors draw attention to the fact that the physical properties of water do not show a uniform change with temperature. There are sudden changes at 15°, 30°, 45° and 60°C, and biological conditions may be particularly unfavourable at these temperatures, whilst optimum conditions might be found at temperatures between those given.

CHAPTER 3

Clinical material

During the period March 1964 to March 1965 *Flavobacterium meningosepticum* was isolated post-operatively from the blood of 10 adult patients admitted to the Department of Thoracic Surgery, Odense County and City Hospital. In nine cases the bacterium was isolated from the blood, and in one case from the sputum.

Technique for blood culture

0.5 ml blood was added to each of the following 12 tubes:

Four tubes with broth containing 1% peptone and 5% horse serum.

Four tubes with broth containing 1% peptone and 0.225% agar.

Four tubes with 1% peptone, 0.225% agar and 0.3% sodium thioglycolate and 5% Fildes extract of horse blood.

The tubes were incubated at 35°C for seven days and inspected daily for growth.

Table 2 contains some of the data from the 10 patients. Patient no. 11 was from a hospital in Copenhagen. The 10 patients from Odense were treated prophylactically with penicillin and streptomycin from the day of operation, whilst patient no. 11 was treated with penicillin, colimycin, Reverin® and Lucopenin® before the bacteraemia was diagnosed as being due to *Flavobacterium meningosepticum*.

In nine of the patients the course was benign. All recovered without specific therapy, as the antibiotics administered have only little effect on *Flavobacterium meningosepticum*. All recovered without sequelae. None of the patients exhibited symptoms or signs from the central nervous system. This course is in contrast to the high mortality rate and the severe sequelae which have been described following meningitis caused by *Flavobacterium meningosepticum* in premature infants.

Patients nos. 9 and 11 died, but in both these patients the associated disease and complications were so serious that it would seem unreasonable to attribute the responsibility for the fatal course to the infection with *Flavobacterium meningosepticum*.

Patient no. 9

In addition to the severe thoracic lesion indicated in Table 2, this patient also had a fractured femur. 10 hours after the operation shown he developed cardiac arrest. Normal cardiac action was rapidly restored by means of external cardiac massage, but during the following two days he developed oliguria with a rapid rise in serum potassium and blood urea, and he was transferred to the dialysis unit of a hospital in Copenhagen. Here numerous colonies of *Flavobacterium meningo-*

Table 2
Data of patients with postoperative Flavobacterium meningosepticum bacteraemia

Patient no.	Sex	Age (yr.)	Diagnosis and operation	Date of operation 1964/65	Interval (hr.)	Hyperpyrexia Date of onset	Duration (hr.)	Max. temp. °C	Date	Blood-culture Positive tubes	Agglutinins
1	M	61	Abdominal aortic aneurysm. Resection and Dacron graft	March 6	30	March 8	50	40.6	March 8	12/12	+
2	F	68	Pulmonary adenomatosis. Segmental resection	April 20	25	April 21	17	41.6	April 21 April 22 April 23	12/12 0/12 0/12	+
3	M	41	Aorto-iliac arteriosclerosis. Bypass reconstruction	April 21	39	April 22	5	40.6	April 23 April 25 April 27	8/12 0/12 0/12	+
4	M	63	Bronchogenic carcinoma. Pneumonectomy	April 28	39	April 29	8	40.5	April 29 10 P.M. April 30 0.30 A.M.	10/12 12/12	+
5	M	32	Stenosis of renal artery. Plastic repair	July 31	50	Aug. 2	10	40.2	Aug. 2 5 P.M. 9 P.M. Aug. 3	0/12 5/12 0/12 0/12	+

6	F	43	Mitral stenosis..... Valvulotomy	Sept. 14	36	Sept. 15	48	40.1	Sept. 15 10 P.M. Sept. 16 0.30 A.M. Sept. 17 Sept. 19	12/12 12/12 7/12 12/12 0/12	+
7	M	68	Gastric carcinoma..... Total gastrectomy	Sept. 28	54	Sept. 30	12	39.7	Sept. 30 8 P.M. Oct. 1	11/12 11/12	-
8	F	48	Cardiospasm..... Heller cardioplasty	Nov. 3	54	Nov. 5	12	39.8	Nov. 5 2.30 P.M. 4 P. M. Nov. 6	0/12 10/12 0/12	+
9	M	19	Bronchial rupture..... Repair	Nov. 14	16	Nov. 15	?	39.6	Nov. 23 Dec. 1	sputum sputum	?
10	M	57	Bronchogenic carcinoma..... Lobectomy	March 9	55	March 11	17	40.0	March 11 March 11 March 12	0/12 1/12 4/12	+
11	F	59	Incontinence of urine..... Marchall-Marchetti repair	Jan. 28 1966	23 days	Feb. 19	10	39.0	Feb. 20	6/6	?

septicum were demonstrated on culture of the sputum 9 and 17 days after the thoracotomy. Cultures were taken from the blood on six occasions and from the peritoneal fluid on three occasions, but the micro-organism was not found in these samples.

The patient died 19 days after operation with uraemia and disseminated *Candida albicans* infection.

Patient no. 11

As mentioned this patient was from a hospital in Copenhagen, but unlike patient no. 9 she had had no contact with Odense County and City Hospital.

The patient was unsuitable for operation because of gross obesity. Two days after operation *Escherichia coli* was demonstrated in the blood, and 14 days later *Staphylococcus aureus* was found. She developed oliguria and azotaemia after repeated episodes of shock associated with fever. The patient died 27 days after operation of uncontrollable rectal haemorrhage. Four days before death *Flavobacterium meningosepticum* was demonstrated in blood cultures. This patient differs from the remainder in the long interval between operation and the *Flavobacterium meningosepticum* bacteraemia.

Bacteriological investigation of autopsy material revealed a few *Klebsiellae* in both lungs. There was no growth from cardiac, splenic or hepatic tissue.

It was characteristic that 1–2 days after a major operation the patients developed a pronounced, but transient, hyperthermia, with little change in their general condition. Fig. 2 shows a typical temperature graph (pt. no. 3). The pulse rate was remarkably low in comparison with the rise in temperature. The normal rise in pulse rate is stated to be about 18 beats per 1°C (Bazett 1924; Lyon 1927; Tanner 1951). Low pulse rates were observed in seven out of the ten patients with bacteraemia, whilst this phenomenon was not observed in patients nos. 1, 4 and 7 (Olsen 1967a).

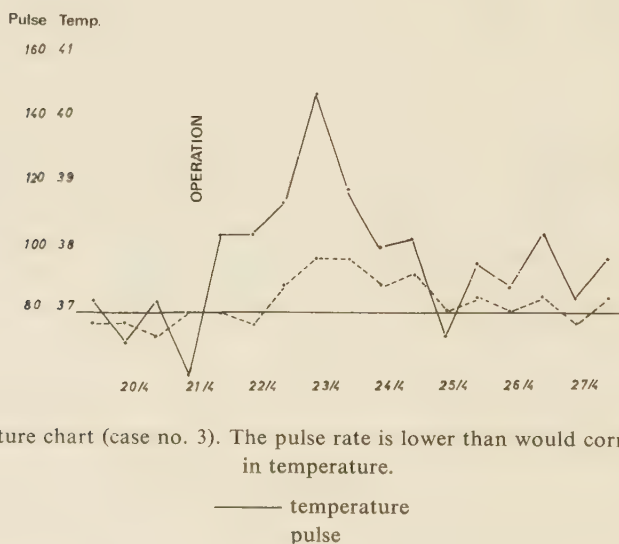


Fig. 2. Temperature chart (case no. 3). The pulse rate is lower than would correspond to the rise in temperature.

— temperature
- - - pulse

The fever lasted for only 1–2 days, which corresponded to the duration of the bacteraemia. In order to establish the diagnosis it is therefore necessary to take blood cultures shortly after the onset of the rise in temperature. On the other hand, the first blood culture was negative in three patients (nos. 5, 8 and 10), such that the rise in temperature had begun before it was possible to demonstrate the bacterium in the blood. It is therefore recommended that repeated blood samples be taken for culture at intervals of a few hours in order to be certain of establishing the diagnosis.

Antibodies

Agglutinating antibodies were demonstrated in the serum of eight patients. The antibody titres varied from 10 to 50 (Olsen et al. 1965). Two patients were not investigated for antibodies, and in one patient (no. 7) no antibodies could be demonstrated. The antibodies appeared 1–2 weeks after the bacteraemia and in one patient were still present 4 months later, whilst in a second patient they disappeared within $2\frac{1}{2}$ months. Four patients were investigated for antibodies within 1–4 days of the onset of the fever, but no antibodies could be demonstrated at that time. When the type giving rise to an epidemic is known, the investigation of serum of the patients for agglutinating antibodies can thus assist in diagnosis, even when the opportunity for bacteriological diagnosis has passed – this has also been described in one case previously (Cabrera & Davis 1961).

Serologically the strain isolated from the patients was found to belong to serotype F (King 1965).

CHAPTER 4

Biochemical properties and dependence of growth on pH and oxygen tension

METHODS

Anaerobic or aerobic growth: Semi-solid agar was inoculated and incubated at room temperature until there was obvious growth (2–3 days).

Motility: Microscopic examination after growth in semi-solid agar and broth.

Catalase reaction: 10% hydrogen peroxide in distilled water.

Oxidase reaction: 1% tetramethyl-para-phenylene diamine in water (Kovacs 1956).

Liquefaction of gelatine, hydrogen sulphide production, reduction of nitrate, Voges-Proskauer's reaction and growth in fluid medium with citrate or glucose: Media and reagents as described by Lautrop (1956). The gelatine tubes were observed for 14 days at room temperature, the nitrate tubes incubated at 35°C for 2 days, and Voges-Proskauer's tubes at 30°C for 2 days. The tubes with citrate or glucose were incubated at 30°C and observed for 14 days.

Growth in potassium cyanide: As described by Møller (1954).

Lysine, arginine and ornithine decarboxylases: As described by Møller (1955).

Indole production: 1% trypsin-digested casein in tap water. Incubation at 35°C for 2 days. The indole was extracted with xylene and Ehrlich-Böhme's reagent added. Purple colouration was indicative of a positive reaction (King 1959).

Urease production: (1) Christensen's substrate (1946). (2) Substrate as (1), but without agar. Incubation at 30°C for 14 days.

Acid production from carbohydrates: Semi-solid agar containing 1% carbohydrate (Hugh & Leifson 1953). Incubation at 35°C for 30 days. Tubes observed daily.

Investigation of growth at different pH: Agar plates adjusted with HCl and NaOH to pH values from 4.5 to 11.0 with intervals of $\frac{1}{2}$ pH.

Ten-fold serial dilutions were made from an overnight broth culture, and from the 6th dilution tube 0.1 ml was spread on each of the agar plates mentioned. This dilution was used because here the number of colonies was countable, and it was thus possible to evaluate the difference in growth at the various pH values within the growth range. The number of colonies was counted after incubation at 35°C for 2 days.

Growth at different oxygen tensions:

1. *High oxygen tension:* 150 ml broth was inoculated with 3 ml of an overnight broth culture grown at 35°C. The mixture was distributed in 500 ml glass flasks, with 20 ml in each, such that the layer of broth comprised 0.5 ml in each flask. The flasks, which were closed with cotton-wool plugs, were then placed on a shaker which

oscillated in the horizontal plane with an amplitude of 8 cm and a frequency of 50/min. The experiment was carried out at room temperature.

The number of bacteria in the shaken flasks was determined at intervals by counting the number of colonies which grew on blood plates after these were inoculated with 0.1 ml of 10-fold dilutions. The oxidation-reduction potential was at the same time determined electrometrically (pH-meter 22, Radiometer A/S, Copenhagen).

2. *Low oxygen tension*: A bacteria culture was produced as described above, but in this experiment it was distributed in WR tubes with rubber corks, with 6 ml in each tube, covered with a 1 cm thick layer of liquid paraffin. The number of bacteria and the oxidation reduction-potential were determined as described above.

Measurement of oxygen tension: The oxygen tension was measured electrometrically in overnight broth cultures covered with a 1 cm thick layer of liquid paraffin. A Clark oxygen electrode E 5046, Radiometer A/S, Copenhagen, was used for the measurements, which were carried out at 38°C.

Strains investigated

The investigation included King's six serological type strains, which were kindly placed at my disposal by the Communicable Disease Center, Atlanta. The strains all originated from premature infants with meningitis.

- A King's no. 14, ATCC 13253
- B King's no. 422, ATCC 13254
- C King's no. 3375, ATCC 13255
- D King's no. 6925
- E King's no. 8388
- F King's no. 8707

In addition, the following seven strains isolated in the Department of Thoracic Surgery, Odense County and City Hospital were also investigated:

Strain no.	Isolated from
5233	Blood from pt. 267/64
6692	Blood from pt. 456/64
6886	Blood from pt. 390/64
T 138	Wash-bowl in anaesthetic preparation room
T 150	Suction apparatus in operating theatre
T 396	Bronchoscopic suction apparatus
L 5	Air in the operating theatre

RESULTS

Flavobacterium meningosepticum is a gram-negative rod which is somewhat polymorphic having cells which vary in size between 1 μ and 3 μ . They are about 0.3 μ thick.

After incubation at 35°C on horse blood agar for one day the colonies measured 1–1.5 mm. There was no haemolysis. On Conradi Drigalski plates the growth was slower, as growth was often not visible until the second day. After incubation at room temperature for 4–5 days on agar plates a yellow pigment was observed, but only in serotypes A, B, D, E and F, and T 150. Formation of pigment was also observed in the growth on semi-solid agar after 1–3 weeks. The consistency of the colonies was butter-like in all except serotype C, where the colonies were very tenacious and elastic.

All strains were found to be *non-motile*, *catalase-positive*, strongly *oxidase-positive* and *aerobic*. It was characteristic by growth in semi-solid agar that in addition to the vigorous growth on the surface there was scanty growth along the stab at a depth of 3–4 cm.

All strains were *indole-positive* and *liquefied gelatine* (1–2 days).

The strains isolated in Odense *split urea* in 2–5 days at 30°C, but not at 37°C. No urease could be demonstrated in the other strains.

The majority of strains *utilized citrate* as sole source of carbohydrate, although this was not true of serotypes A, C, E and F.

No strain reduced *nitrate*, and none grew in *potassium cyanide*. All strains were *Voges-Proskauer-negative* and none produced *lysine*, *arginine* or *ornithine decarboxylases*. In no case was *hydrogen sulphide* produced.

Acid production from carbohydrates, alcohols and glycosides

In all strains acid production was oxidative.

The following carbohydrates and alcohols were oxidized within 4 days: glucose, laevulose, maltose, trehalose, mannitol, glycerol and ethyl alcohol.

Galactose was oxidized by all strains within 3–4 weeks.

All strains produced acid from lactose within 30 days, but the time of the positive reaction showed great variation. Serotypes A and F were the quickest (2–3 days), whilst serotypes C, D and E were slower (2–3 weeks). In the remaining strains the reaction became positive during the 4th week.

The following carbohydrates were not oxidized within 30 days: 1-arabinose, xylose, rhamnose, sucrose, raffinose, starch, inulin, adonitol, sorbitol, dulcitol, inositol and salicin.

Exceptions: T 150 did not oxidize ethyl alcohol. Serotypes D and E oxidized xylose within 4–7 days. Serotype F oxidized arabinose within 7 days.

Growth at different pH

In addition to the 13 strains of *Flavobacterium meningosepticum* already mentioned, the following six strains were investigated for purposes of comparison:

Pseudomonas aeruginosa ATCC 10145.

Pseudomonas fluorescens V 94, isolated from the water supply in Odense; identified as biotype 63 by Jessen's method (1965).

Staphylococcus aureus 25157, isolated from pus at Statens Seruminstitut, Regional Laboratory, Odense.

Escherichia coli SS.C 06 Bi 7458/41.

Proteus rettgeri Å 104, isolated from Odense River.

Enterobacter cloaca 7669, isolated from a cerebral abscess at Statens Seruminstitut, Regional Laboratory, Odense (Jøker et al. 1965).

The results of the pH investigations are shown in Table 3. The 13 strains of *Flavobacterium meningosepticum* all gave the same result, and are therefore shown together. On the plates with growth the number of colonies was approximately the same at all pH values, although for *Pseudomonas aeruginosa* there was some reduction of growth at the most basic levels, both the number and size of the colonies being smaller. The same was true of *Staphylococcus aureus* at the most acid levels.

Table 3

	Agar plates adjusted to the following p _H -values												
	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0
<i>Flavobacterium meningosepticum</i>	—	—	+	+	+	+	+	+	—	—	—	—	—
<i>Staphylococcus aureus</i>	—	(+)	(+)	+	+	+	+	+	—	—	—	—	—
<i>Pseudomonas fluorescens</i>	—	—	+	+	+	+	+	+	—	—	—	—	—
<i>Pseudomonas aeruginosa</i>	—	+	+	+	+	+	+	+	(+)	(+)	—	—	—
<i>Escherichia coli</i>	—	—	+	+	+	+	+	+	+	+	+	+	—
<i>Enterobacter cloaca</i>	—	+	+	+	+	+	+	+	+	+	+	+	—
<i>Proteus rettgeri</i>	—	+	+	+	+	+	+	+	+	+	+	+	—

Growth of six different bacteria on agar plates adjusted to different p_H-values between 5.0–11.0. + indicates growth, (+) reduced growth, — no growth.

Flavobacterium meningosepticum grew in the interval pH 6.0–8.5. The same was true of *Pseudomonas fluorescens* and *Staphylococcus aureus*, whilst *Pseudomonas aeruginosa* also grew at pH 9.5.

The three enterobacteria studied were better able to tolerate a basic environment than the remainder.

Growth at different oxygen tensions

The following strains, of those mentioned above, were studied:

Flavobacterium meningosepticum 5233.

Pseudomonas aeruginosa ATCC 10145.

Escherichia coli SS.C 06 Bi 7458/41.

The results are apparent from Figs. 3, 4 and 5. The time in hours is shown on the abscissa, and the oxidation-reduction potential (E_h) and the logarithm of the number of bacteria (N) are shown on the ordinate.

In *Flavobacterium meningosepticum* and *Pseudomonas aeruginosa* it was found that during growth under aerobic conditions (agitating of culture) the exponential phase

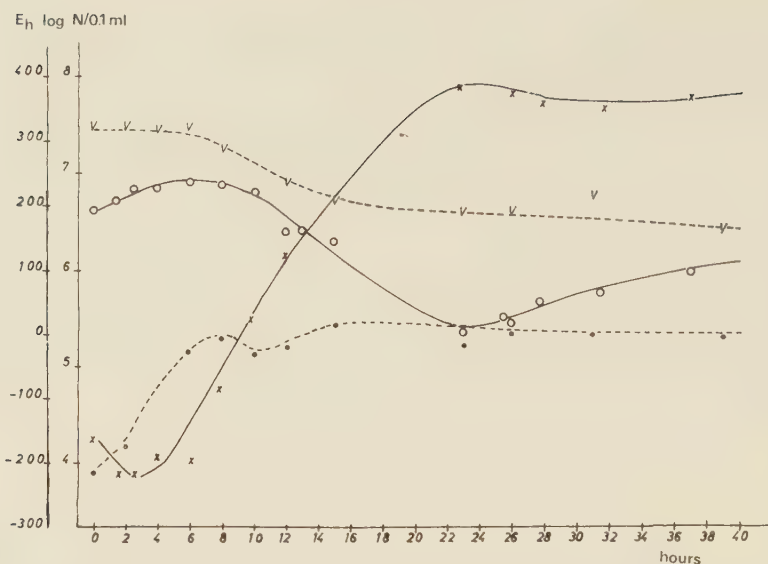


Fig. 3. *Flavobacterium meningosepticum* 5233.

Number of bacteria and oxidation-reduction potential as a function of time during growth in broth under aerobic and anaerobic conditions.

- x—x number of bacteria per 0.1 ml under aerobic conditions
-● number of bacteria per 0.1 ml under anaerobic conditions
- oxidation-reduction potential under aerobic conditions
- v.....v oxidation-reduction potential under anaerobic conditions.

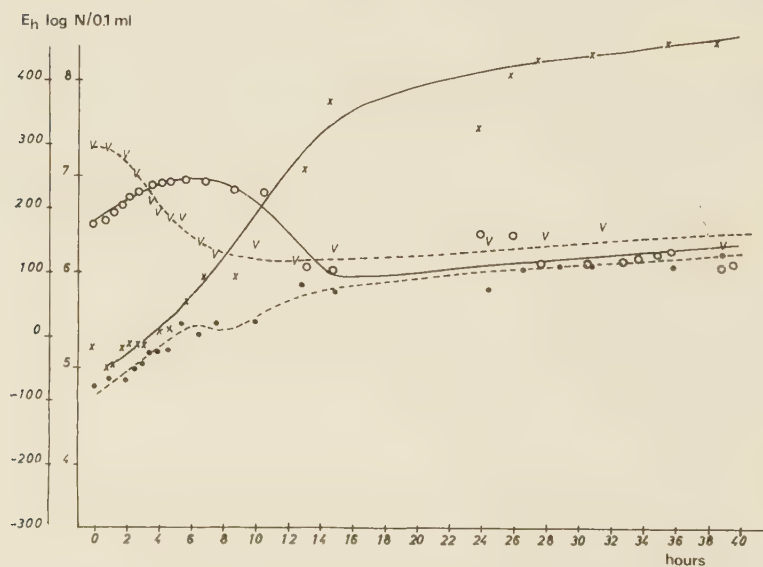


Fig. 4. *Pseudomonas aeruginosa* ATCC 10145.

Legend see fig. 3.

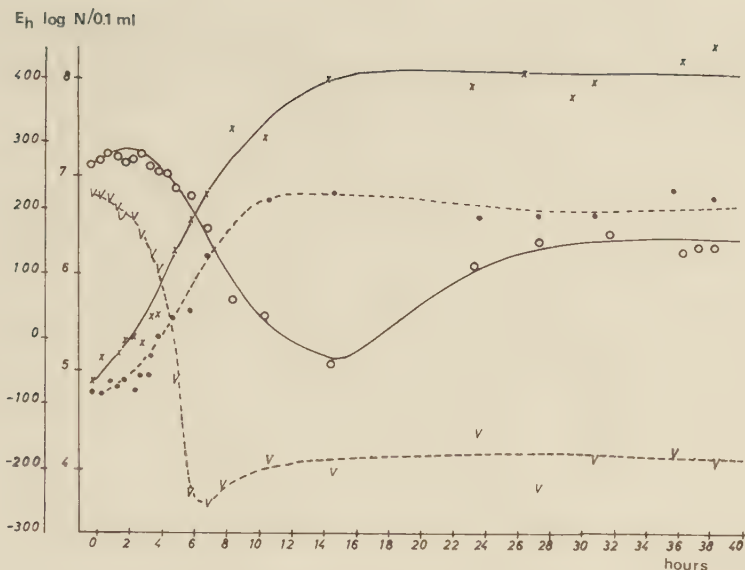


Fig. 5. *Escherichia coli* SS.C 7458/41.

Legend see fig. 3.

was obviously longer than during growth under anaerobic conditions (culture covered with liquid paraffin). This meant that the final number of bacteria was greater under aerobic conditions. This phenomenon was less pronounced in *Escherichia coli*. This has been demonstrated previously for *Pseudomonas aeruginosa* (Owen & Hill 1965).

Under both aerobic and anaerobic conditions all three bacteria showed a fall in oxidation-reduction potential. The end of this fall coincided with the end of the exponential phase. After the fall the oxidation-reduction potential remained almost constant under anaerobic conditions, but under aerobic conditions the fall was replaced by a rise. This was less marked for *Pseudomonas aeruginosa* than for the other two species.

Measurements of oxygen tension

Measurements of the oxygen tension after 24 hours of growth in broth covered with liquid paraffin were carried out on 14 strains of *Flavobacterium meningosepticum*, nine strains of *Pseudomonas aeruginosa* and the strain of *Escherichia coli* already named.

In 12 of the strains of *Flavobacterium meningosepticum*, six of the strains of *Pseudomonas aeruginosa* and the *Escherichia coli*, the oxygen tension was found to 0 mm. One strain of *Flavobacterium meningosepticum* and one of *Pseudomonas aeruginosa* had oxygen tensions of 1 mm, and two strains of *Pseudomonas aeruginosa* and one of *Flavobacterium meningosepticum* had oxygen tensions of 3 mm.

As these low oxygen tensions lie within the range of experimental error for the method of measurement used, this indicates that all the strains studied have used all the free oxygen to be found in the broth substrate.

DISCUSSION

One unusual property of *Flavobacterium meningosepticum* is its ability to produce indole, which is otherwise a characteristic of bacteria which reduce nitrate and produce acid anaerobically from carbohydrates (Leifson 1966).

Flavobacterium meningosepticum was frequently isolated from the water in the air-conditioning apparatus connected with the operation theatres. The water here is often alkaline, as the humidifiers use ion-exchanged water which in intimate contact with air releases carbon dioxide from the dissolved bicarbonate; pH values of up to 9.3 have been measured electrometrically. It was therefore to be assumed that the micro-organism was resistant to alkali, and that it would be possible to use a solid substrate with alkaline reaction for the selective isolation of this bacterium. However, the present investigation has revealed that *Flavobacterium meningosepticum* tolerates the alkaline reaction of this type of substrate no better than *Staphylococcus aureus* or *Pseudomonas*, and that it does not tolerate it as well as the enterobacteria (Table 3, p. 31).

Staphylococcus aureus is stated to grow in the pH interval 6–8 (Dernby 1921; Peterson et al. 1964). The interval for *Pseudomonas aeruginosa* is given as 5.6–8 (Dernby 1921) and that for *Pseudomonas fluorescens* as 5.4–8.85 (Wodinsky & Frazier 1960). These results correspond approximately to those found in the present study. In contrast *Escherichia coli* has been found in the present investigation to be more resistant to alkali than stated by other workers, who have found growth in the interval 4.5–9.0 (Dernby & Näslund 1923; Gale & Epps 1942). This difference may be due to the solid medium employed in this investigations, as the pH in the bacterial cell in itself depends, in addition to the pH in the macro-environment, on the permeability of the cell membrane to hydrogen ions, and this may be affected by the composition of the substrate.

The ability to grow in the depths of the medium after stab inoculation in semi-solid agar could perhaps suggest that with regard to oxygen dependence *Flavobacterium meningosepticum* occupies a position between a strictly aerobic bacterium such as *Pseudomonas aeruginosa*, and an anaerobe such as *Escherichia coli*. The present investigation has demonstrated that in this respect *Flavobacterium meningosepticum* most closely resembles *Pseudomonas aeruginosa*, as growth in both these bacteria is promoted by high oxygen tension to a greater extent than the growth in *Escherichia coli*. However, all three bacteria are capable of utilizing practically all the available oxygen in a broth substrate.

The ability to utilize the oxygen in a substrate has been investigated in only a small number of bacteria. Longmuir (1953) used a refined electrometric technique to

determine the oxygen concentration at which the respiratory rate was half of the maximum in a number of bacteria. Both aerobic (gram-positive rods) and anaerobic bacteria (enterobacteria) were included in the investigation. The values found varied from $1.01 \cdot 10^{-8}$ M to $1.57 \cdot 10^{-6}$ M. Aerobic bacteria were thus capable of utilizing oxygen even at these low oxygen concentrations. The highest values were found for the aerobic bacteria, but as these bacteria were also the largest, the author considers that this finding was due to the greater difficulty in diffusion of oxygen in these bacteria.

The changes in oxidation-reduction potential in the various bacteria during growth are not directly comparable, as, in addition to the type of bacteria, such changes are also highly dependent on the substrate. The age of the substrate, its oxygen and glucose contents will thus affect the oxidation-reduction potential, whilst the same is true of the addition of serum (Hewitt 1950). Small differences must therefore be accepted with reservations. However, in the present investigation there is no doubt that under anaerobic conditions there was a greater fall in oxidation-reduction potential for *Escherischia coli* than for the other two bacteria, whilst under aerobic conditions the fall was perhaps slightly less for *Pseudomonas aeruginosa* than for the other two. It was true of all three bacteria that the fall in oxidation-reduction potential was associated with the exponential phase, as the end of this coincided with the minimum oxidation reduction-potential.

The importance of temperature for the growth of *Flavobacterium meningosepticum*

Clinically, bacteraemia with *Flavobacterium meningosepticum* in adults was characterized by a pronounced but transient hyperthermia associated with a benign course, with recovery without any specific therapy.

One possible explanation of the spontaneous recovery in connection with hyperthermia might be that *Flavobacterium meningosepticum* is a thermolabile micro-organism. In order to investigate this, the growth of *Flavobacterium meningosepticum* in vitro was studied at a number of temperatures between 4° and 41°C.

METHODS

1. *Determination of the generation time*

The generation time was determined from the exponential part of the growth curve, which in turn was found by removing 0.1 ml of a growing broth culture at intervals, using these samples to produce ten-fold serial dilutions, and inoculating 0.1 ml of each of these on blood agar plates. The plates were incubated at 35°C for 2 days, and the colonies counted.

If the results are shown graphically with the time in hours as abscissa and the logarithm of the bacterial count as ordinate, the generation time may be calculated from the equation

$$\alpha = \frac{\log N_2 - \log N_1}{t_2 - t_1} = \frac{\log 2}{g}$$

where α is the slope of the rectilinear part of the graph, N_1 and N_2 the bacterial counts at times t_1 and t_2 , and g is the generation time.

2. *Titration of the 50 per cent end-point (ID 50)*

Ten-fold serial dilutions in nine tubes were made from an overnight broth culture incubated at 35°C. 0.1 ml from each tube in the series was dropped into five tubes containing semi-solid agar, such that 45 tubes were used in each experiment. The inoculated agar tubes were incubated at the appropriate temperatures and the number of tubes containing visible growth was noted daily. All tubes were observed for one week, with the exception of those incubation at 10°C where growth is slower, and these tubes were therefore observed for 12 days. Kärber's method was used to determine the 50 per cent end-point (Mackie & McCartney 1962).

The investigations at 37°, 38°, 39°, 40° and 41°C were carried out in a water bath, the temperature of which was regulated with an accuracy of 0.1°C, whilst those at 4°, 10°, 30° and 35°C were carried out in an air thermostat with an accuracy of 1°C. Finally, experiments were carried out at room temperature, which varied between 21° and 24°C. In all investigations a control experiment was carried out at 30°C using the same ten-fold dilutions.

All the 13 strains of *Flavobacterium meningosepticum* named in Chapter 4 were included in the study.

RESULTS

Method 1

Using this method bacteraemia strain 5233 was investigated at 21° and 35°C. The temperature was regulated with the water bath thermostat.

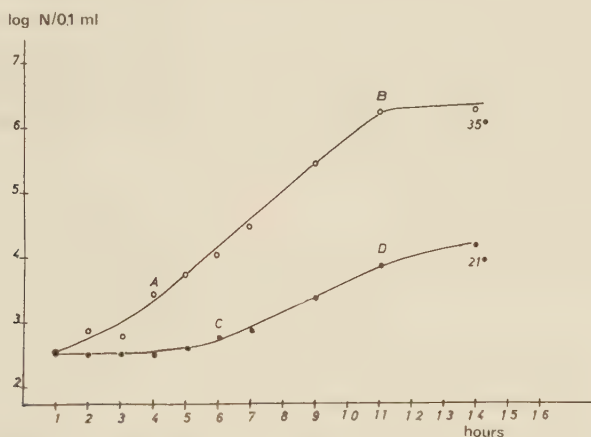


Fig. 6. Growth graphs for bacteraemia strain 5233 at 21° and 35°C. The rectilinear parts of the two graphs were used for calculation of the generation times at the two temperatures.

The results may be seen from Fig. 6. A and B have coordinates (4.0, 3.5) and (11.0, 6.3) and C and D coordinates (6.0, 2.8) and (11.0, 3.9). From the equation given it is found that

the generation time at 35°C = 0.75 hours

the generation time at 21°C = 1.36 hours.

Flavobacterium meningosepticum is thus a slowly growing bacterium.

Method 2

At 4° and 41°C there was no growth of any strain. The results at the temperatures 35°, 37° and 38°C are shown in Tables 4, 5 and 6. The name of the strain is shown in the column on the left, and in the column on the extreme right is shown the maximum titre reached in the control experiment carried out at the same time at 30°C. At 30°C

Table 4
Results at 35°C

Strain	log ID50							log ID50 at 30°C
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
King A.....	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1
King B.....	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.1
King C.....	8.1	8.3	8.3	8.3	8.3	8.3	8.3	8.5
King D.....	7.3	7.7	7.7	7.7	7.7	7.7	7.7	8.1
King E.....	6.3	7.3	7.3	7.3	7.3	7.3	7.3	6.7
King F.....	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.7
5233.....	6.5	7.3	7.3	7.3	7.3	7.3	7.3	7.3
6886.....	6.9	7.5	7.5	7.5	7.5	7.5	7.5	7.5
6692.....	7.7	8.1	8.1	8.1	8.1	8.1	8.1	8.3
T 138.....	7.1	7.3	7.3	7.3	7.3	7.3	7.3	7.1
T 150.....	6.7	7.5	7.5	7.5	7.5	7.5	7.5	7.3
T 396.....	7.9	8.1	8.1	8.1	8.1	8.1	8.1	8.3
L 5.....	6.3	6.9	6.9	6.9	6.9	6.9	6.9	6.7

Growth of 13 *Flavobacterium meningosepticum* strains at 35°C using method 2. The titres obtained at 30°C are shown in the column furthest to the right. There is no difference between the strains, and the growth is equal at 30° and 35°C.

For details of method see p. 36.

Table 5
Results at 37°C

Strain	log ID50							log ID50 at 30°C
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
King A.....	7.7	7.9	7.9	7.9	7.9	7.9	7.9	8.1
King B.....	7.9	8.1	8.1	8.1	8.1	8.1	8.1	8.1
King C.....	8.3	8.9	8.9	8.9	8.9	8.9	8.9	9.1
King D.....	7.3	7.3	7.3	7.3	7.3	7.3	7.3	8.1
King E.....	6.5	6.7	6.7	6.7	6.7	6.7	6.7	6.7
King F.....	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.7
5233.....	2.5	3.9	4.5	5.3	5.3	5.3	5.3	7.3
6886.....	3.5	5.3	6.1	6.1	6.1	6.1	6.1	6.9
6692.....	1.5	4.9	5.7	5.9	5.9	5.9	5.9	7.1
T 138.....	1.5	2.5	3.3	3.3	3.3	3.3	3.3	7.7
T 150.....	1.5	1.5	1.5	2.3	2.3	2.3	2.3	8.1
T 396.....	<0.5	1.3	2.9	3.1	3.5	3.5	3.5	7.3
L 5.....	1.5	3.3	4.5	4.5	4.5	4.5	4.5	6.7

The meningitis strains (King) grew equally well at 30° and 37°C, while the growth of the remaining strains was inhibited at 37°C; this is apparent from the low titres on the first days and the finding that the maximum titres at 30° were not reached.

For details of method see p. 36.

Table 6
Results at 38°C

Strain	log ID50							log ID50 at 30°C
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
King A.....	7.1	7.9	7.9	7.9	7.9	7.9	7.9	8.1
King B.....	7.5	8.1	8.1	8.1	8.1	8.1	8.1	8.1
King C.....	6.9	8.3	8.3	8.3	8.3	8.3	8.3	8.5
King D.....	6.7	7.1	7.1	7.1	7.1	7.1	7.1	6.5
King E.....	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.7
King F.....	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.5
5233.....	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	7.3
6886.....	0.7	0.7	0.7	0.7	0.7	0.7	0.7	7.9
6692.....	1.5	1.5	1.5	1.5	1.5	1.5	1.5	8.3
T 138.....	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	6.7
T 396.....	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	6.9
L 5.....	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	7.3

The meningitis strains (King) grew equally well at 30° and 38°C but the remaining strains grew only a little or not at all at 38°C.

For details of method see p. 36.

this maximum titre was in the majority of cases reached after one day, and in all cases after two days. The results at the remaining temperatures investigated are given in an earlier publication (Olsen 1966).

At 38°C the strains clearly separate into two groups, as may be seen from Table 6. The meningitis strains (King) continued to grow at this temperature as they had done at 30°C, whilst the bacteraemia strains (5233, 6886 and 6692) and three strains isolated from the environment showed very little growth.

The growth of these latter strains was already retarded at 37°C, as the titres on the first days were low (Table 5). However, in contrast to the strains isolated from the environment, the bacteraemia strains eventually reached titres in the neighbourhood of the maximum reached at 30°C.

At room temperature (21–24°C) growth in all strains was somewhat slower than at 30°C, there being some reduction in titre on the first day. This corresponds to the longer generation time which was demonstrated by method 1. At 10°C this phenomenon was considerably more pronounced. However, both at room temperature and at 10°C the maximum titres obtained at 30°C were reached, after two days at room temperature, but not until after 6–10 days at 10°C (Olsen 1966).

At 39°C the meningitis strains still grew as readily as at 30°C, although there was perhaps some slight reduction in titre during the first days, but at 40°C the titres during the first days were definitely lower. King's serotype E showed the greatest inhibition of growth (Olsen 1966).

At 30° and 35°C growth in all strains seemed to be uniform, and statistically no difference was found between the titres at these two temperatures.

If the maximum titres at these temperatures (Table 4, p. 38) are used to calculate the differences

$d_1 = \log \text{ID}_{50_{35^\circ}} - \log \text{ID}_{50_{30^\circ}}$, it can be calculated that

$$\bar{d} = 0.046 \quad s = 0.28 \quad e = \frac{s}{\sqrt{n}} = 0.08 \quad t = \frac{\bar{d}}{e} = 0.58.$$

As there is no difference between the titres at these two temperatures, they can be considered as duplicate determinations at the optimum growth temperature, and as an expression of the error for the individual titration at optimum temperature one obtains

$$s_r = \sqrt{\frac{\sum d_i^2}{2k}} = 0.196, \text{ where } k \text{ is the number of duplicate determinations, in this case } 13.$$

From this it is possible to calculate the maximum deviation $= 2.58\sqrt{2s_r^2} = 0.72$ ($P < 0.01$).

Thus if there is a difference of more than 0.72 $\log_{10}$ units in the results of simultaneous titrations of a culture at two different temperatures, one of which is optimum, then the other is not optimum.

Regular microscopic examinations of the cultures were carried out during the investigation. At critical growth temperatures the morphology of the micro-organism was altered, such that it became more polymorphic, with long, filiform elements. In the meningitis strains this phenomenon was observed at 40°C, whilst it was already visible at 37°C in the remaining strains. At 10°C, where the rate of growth was obviously reduced, the morphology was normal. Thus microscopy alone was sufficient to determine whether or not there was reduction of growth at a given temperature.

The sensitivity of Flavobacterium meningosepticum to temperatures above the maximum growth temperature

Technique

Using the experimental set-up described earlier six sets of 45 tubes containing semi-solid agar were inoculated with King's serotype C, and six sets with bacteraemia strain 5233. The six sets containing serotype C were incubated at 41°C, and those with 5233 at 38°C. One set of each was removed daily; this was then incubated at 30°C for 7 days with daily determinations of titre.

After incubation at these temperatures for one day, serotype C showed a fall in titre of 5.4, and 5233 a fall of 3.4, corresponding to a considerable reduction in the bacterial count. After 2 days of incubation at 41°C there was no growth of serotype C, and after 4 days of incubation at 38°C there was no growth of 5233 (Olsen 1966).

This investigation has revealed an obvious difference between the strains isolated from premature infants with meningitis and those isolated in Odense County and City Hospital. The results obtained for the meningitis strains more or less correspond to those reported by other workers (King 1959; Buttiaux & Vandepitte 1960; Sugathadasa & Arseculeratne 1963).

The bacteraemia strains already showed inhibition of growth at 37°C, and were there-

fore more thermolabile than the majority of pathological bacteria (see Table 1, p. 18). The thermolability of these strains gives a satisfactory explanation of the finding that adult patients with bacteraemia recover spontaneously in association with the pronounced rise in temperature.

The strains isolated from premature infants with meningitis were less thermolabile, and this would explain their greater pathogenicity.

The fact that the more thermoresistant strains are isolated from geographical regions with hot climates, whilst the thermolabile strains have been isolated in the temperate zone may perhaps be associated with the difference in sensitivity to temperature which has been described for other micro-organisms earlier in this monograph (p. 20).

CHAPTER 6

The sensitivity of *Flavobacterium meningosepticum* to antibiotics

The investigation comprised the 13 strains of *Flavobacterium meningosepticum* named in Chapter 4 (p. 29).

METHODS

1. Agar diffusion method using tablets (*Sensitabs*® (Rosco))

The culture was spread onto 10% horse blood agar without peptone containing 1% glucose (Thomsen 1967). Immediately afterwards the Sensitabs were placed on the plates, and the zones without growth were measured after incubation at 35°C overnight.

The Sensitabs were 9 mm in diameter and contained the following amounts of antibiotic:

erythromycin	500 µg	tetracycline	1 mg
novobiocin	10 mg	ampicillin	100 µg
fucidin	4 mg	chloramphenicol	500 µg
neomycin	6 mg	streptomycin	1 mg
vancomycin	1 mg	kanamycin	1 mg
penicillin	15 µg	colimycin	5 mg
methicillin	50 mg	sulphamethizole	10 mg
bacitracin	10 mg	nalidixic acid	5 mg

The manufacturer's instructions give the relation between the extent of the zone and the four degrees of sensitivity shown below. It is necessary to divide the Sensitabs into three groups, A, B and C, where A consists of vancomycin, B of bacitracin and colimycin, and C of the remaining antibiotics.

Degree of sensitivity	A	B	C
Sensitive	≥ 18 mm	≥ 22 mm	≥ 28 mm
Moderately sensitive	15–17 mm	20–21 mm	23–27 mm
Relatively resistant	10–14 mm	10–19 mm	10–22 mm
Resistant	≤ 9 mm	≤ 9 mm	≤ 9 mm

2. Tube dilution method with determination of the 50 per cent inhibitory concentration (IC 50)

Two 10-fold serial dilutions of an antibiotic dissolved in broth were made, with 0.5 ml in each tube and as a rule 1000 µg/ml in the first tube. The tubes were inoculated

with a platinum loop and incubated at 35°C for 2 days. Blood agar plates were inoculated from all tubes without visible growth, in order to determine whether the action of the antibiotic was bacteriocidal or bacteriostatic.

From the number of tubes with visible growth a titre ($\log_2$ IC 50) was calculated by Kärber's method (Mackie & McCartney 1962), and from this the IC 50 was calculated by means of the equation

$$\text{IC 50} = \frac{1000}{2^{10-T}} \sim 2^T$$

where T is the titre found when the initial tube is counted as 10 and contains 1000 µg/ml antibiotic.

The inoculum, which was determined in every test by counting on blood agar plates as described in Chapter 5 (p. 36), varied between 10^4 and 10^5 per tube.

3. *Combination of two antibiotics*

It may be assumed that it is not possible to maintain therapeutic serum concentrations of antibiotics exceeding 20 µg/ml, and in order to limit the number of experiments it was therefore considered that any antibiotic which did not have a synergistic action with another antibiotic at this concentration was of no therapeutic interest, and such combinations were not investigated further.

Based on this prerequisite, the following experimental programme was set up:

a. *Combination of two antibiotics with IC 50 > 40 µg/ml*

If the antibiotics which are to be studied in pairs are called A, B and C, then the tubes are set up according to the following system (Garrod & Waterworth 1962):

AA	AB	AC
	BB	BC
		CC

In accordance with the prerequisite mentioned above the tubes of type AB contained 20 µg/ml of antibiotic A + 20 µg/ml of antibiotic B. Tubes of type AA contained 40 µg/ml of antibiotic A. The tubes were inoculated and incubated at 35°C for 2 days. There will always be growth in tubes of type AA. If there is no growth in tubes of type AB this indicates that the two antibiotics have a potentiating action. Such combinations have been investigated in more detail as described under method c, below.

b. *Combination of antibiotics with IC 50 > 40 µg/ml with an antibiotic with IC 50 < 40 µg/ml*

A number of two-fold serial dilutions of an antibiotic A with IC 50 < 40 µg/ml were prepared. One duplicate series contained only antibiotic A, whilst 20 µg/ml of all

the antibiotics investigated with $IC_{50} > 40 \mu\text{g/ml}$ were added to the other duplicate series. The tubes were inoculated and incubated at 35°C for 2 days. If the addition of an antibiotic B leads to a fall in titre, there must be synergism between A and B, whilst a rise in titre indicates antagonism between A and B.

A fall in titre of one $\log_2$ unit means that the $20 \mu\text{g/ml}$ of antibiotic B which was added substituted up to 50% of the IC_{50} of antibiotic A. A fall in titre of more than one $\log_2$ unit means that there is a potentiating effect.

All the combinations of antibiotics in which there was a fall in titre after incubation for 2 days were further investigated by the method described under c.

c. *Combination of two antibiotics with $IC_{50} < 40 \mu\text{g/ml}$*

Two-fold serial dilutions in broth of the two antibiotics which were to be combined were prepared. 64 tubes were thereafter set up in a chess-board pattern. The tubes in the row on the extreme right contained only one of the antibiotics in two-fold dilutions, and in the lowest horizontal row there was correspondingly only the other antibiotic. The remaining tubes contained the two antibiotics in all the concentrations which could be obtained by a combination of the two serial dilutions. The tubes were inoculated and incubated at 35°C for 2 days, after which the number of tubes with visible growth was recorded.

If the initial concentrations are suitable, then zones without growth should be present on the left and upper parts of the system, as it is attempted to make the antibiotic concentrations in these areas higher than the least inhibitory concentration of the two antibiotics. In the following figures these areas are to be seen above and to the left of the dotted lines. If tubes with growth are encountered within these areas, this indicates antagonism between the two antibiotics (Fig. 8, p. 48). If there are tubes without growth below and to the right of these lines, there is synergism, and the number of tubes in which there is no growth will be a measure of the degree of synergism. If there is a tube without growth in which both antibiotics are diluted by at least two steps from their least inhibitory concentration, this indicates a definite potentiating effect. (Fig. 7, p. 48).

Blood agar plates were spread from the tubes without visible growth in order to obtain an expression of the bacteriocidal effect of the combination.

4. *Investigation for penicillinase production*

Agar plates containing 1% starch were inoculated by stabbing. After overnight incubation a solution of 60 mg benzyl-penicillin dissolved in 1 ml water was poured over, and the plates were left at 4°C for 30 minutes. After this an iodine-potassium iodide solution was added. Where there had been penicillinase production a clear zone appeared around the colonies after a few minutes. This contrasted with the blue colouration of the remainder of the plate (Perret 1954).

All the 13 strains of *Flavobacterium meningosepticum* investigated produced penicillinase.

5. Investigation of the importance of the inoculum for penicillin sensitivity

As *Flavobacterium meningosepticum* produces penicillinase it might be expected that the sensitivity to penicillin would be decreased if the inoculum were increased. This has been demonstrated for penicillinase-producing staphylococci (Barber 1947; Eriksen & Erichsen 1964). This was investigated by the following technique (Seligman 1966):

Agar plates containing two-fold serial dilutions of benzyl-penicillin were spread with various inocula. One serial dilution was spread with 0.1 ml of an undiluted overnight broth culture, and three others with 0.1 ml of the same culture diluted 10^2 , 10^4 and 10^6 times.

In order to induce an increase in penicillinase production the experiment was also carried out using a culture which had been grown in two passages lasting one day in broth containing 10 µg/ml penicillin.

RESULTS

Tables 7 (p. 46) show the results of the agar diffusion method using Sensitabs® (Rosco).

Table 8 (p. 47) shows IC 50 determined by the tube dilution method. Each result is based on at least two determinations of $\log_2$ IC 50, and the mean has been used to calculate the IC 50 according to the formula given in the description of the method (p. 43). A total of 328 determinations were carried out. The standard deviation of $\log_2$ IC 50 has been calculated to be 0.81 (Olsen 1967c).

The following antibiotics were found to be bacteriocidal: vancomycin, penicillin, ampicillin and streptomycin, whilst the remainder had a bacteriostatic action. Even at high concentrations of novobiocin there were numerous surviving colonies, whilst all the remaining bacteriostatic antibiotics had a bacteriocidal action in concentrations of twice to 4 times the inhibitory concentration.

Table 9 (p. 47) contains the results observed after 2 days in the combination experiments using the three methods described (pp. 43–44). These tests were carried out using King's serotype C, which is the type most commonly isolated from premature infants with meningitis.

The most marked synergism was found for the combination erythromycin/pyrrolidino-methyltetracycline, but even here the synergism was, however, quite weak. The results of the test of this combination carried out by method 3c are shown in Fig. 7 (p. 48), which indicates that there was a potentiating effect.

Antagonism has been demonstrated in the combinations vancomycin/ampicillin (Fig. 8, p. 48) and vancomycin/penicillin, and also for novobiocin/sulphadimidine (Table 9, p. 47).

If the bacteriocidal action of the combination is evaluated by inoculating from the tubes without growth, the synergism is found to be less pronounced.

Using method 3c it was found that for the combination erythromycin/penicillin there were a few tubes in which there was growth despite the fact that the penicillin

Table 7

	King A	King B	King C	King D	King E	King F	5233	6692	6886	T 150	T 138	T 396	L 5
Vancomycin.....	25 ++	25 ++	25 ++	25 ++	25 ++	25 ++	23 ++	24 ++	22 ++	25 ++	25 ++	24 ++	24 ++
Fucidin.....	31 ++	31 ++	31 ++	31 ++	31 ++	31 ++	31 ++	32 ++	28 ++	33 ++	34 ++	34 ++	34 ++
Erythromycin.....	32 ++	32 ++	31 ++	34 ++	32 ++	36 ++	35 ++	30 ++	32 ++	33 ++	25 ++	38 ++	25 ++
Novobiocin.....	26 ++	30 ++	31 ++	30 ++	30 ++	29 ++	35 ++	36 ++	29 ++	30 ++	32 ++	34 ++	31 ++
Neomycin.....	22 ++	20 ++	29 ++	25 ++	29 ++	27 ++	26 ++	28 ++	31 ++	27 ++	30 ++	30 ++	30 ++
Nalidixan.....	24 ++	29 ++	28 ++	25 ++	26 ++	22 ++	24 ++	26 ++	24 ++	21 ++	28 ++	22 ++	26 ++
Chloramphenicol.....	19 ++	22 ++	20 ++	21 ++	19 ++	27 ++	15 ++	19 ++	18 ++	16 ++	14 ++	17 ++	15 ++
Streptomycin.....	21 ++	20 ++	21 ++	21 ++	23 ++	19 ++	15 ++	19 ++	19 ++	12 ++	14 ++	18 ++	14 ++
Tetracycline.....	12 ++	20 ++	15 ++	13 ++	17 ++	15 ++	12 ++	12 ++	12 ++	12 ++	14 ++	12 ++	13 ++
Kanamycin.....	12 ++	12 ++	17 ++	14 ++	17 ++	15 ++	12 ++	16 ++	15 ++	0 ++	0 ++	13 ++	0 ++
Bacitracin.....	0 ++	0 ++	15 ++	14 ++	15 ++	14 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++
Penicillin.....	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++
Methicillin.....	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++
Ampicillin.....	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++
Colimycin.....	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++
Sulphamethizole.....	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++	0 ++

Determination of the sensitivity of 13 *Flavobacterium meningosepticum* strains to 16 antibiotics using an agar diffusion method. The figures indicate the inhibition zones in millimetres, ++ sensitive, +++ moderately sensitive, + relatively resistant, 0 resistant.

Table 8

	IC 50 µg/ml range	IC 50 µg/ml mean
Erythromycin.....	1.7-16	5.3
Novobiocin.....	2.0-36	8.0
Vancomycin.....	6.8-38	17
Fucidin.....	5.0-54	23
Penicillin.....	24-95	51
Chloramphenicol.....	14-88	61
Tetracycline.....	44-107	70
Ampicillin.....	47-154	107
Streptomycin.....	102-574	274
Sulphadimidine.....	1516-6061	3407
Polymyxin B.....	12790-36180	19490

Determination of sensitivity of 11 *Flavobacterium meningosepticum* strains to 11 antibiotics using a tube dilution method (p. 42). (Olsen 1967 c).

Table 9

	Erythro- mycin	Novo- biocin	Vanco- mycin	Fuci- din	Peni- cillin	Chlor- amphe- nicol	Tetra- cycline	Ampi- cillin	Strepto- mycin
Novobiocin.....	S								
Vancomycin.....	S	S							
Fucidin.....	S	O**	S						
Penicillin.....	O	S	A	S					
Chloramphenicol.....	S	O**	S	S	O				
Tetracycline.....	S	O**	O**	O*	O	O*			
Ampicillin.....	O**	O**	A	O*	O**	O*	O*		
Streptomycin.....	O**	O**	O**	O*	S	O*	O*	O*	
Sulphadimidine.....	O**	A**	O**	O*	O**	O*	O*	O*	O*

Flavobacterium meningosepticum (King's serological type C).

Results obtained by a combination of two antibiotics using the three methods described. Results obtained by method 3a are marked *, for those using method 3b ** is used. No mark indicates that method 3c was used.

S: synergism, A: antagonism, O: indifference.

The methods are described on pp. 43-44.

concentration in these tubes was above the minimum inhibitory concentration. This might have been due to a weak antagonism. However, on supplementary investigations using a modification of Elek & Hilson's technique (Dons 1966) it was not possible to demonstrate any antagonism between these two antibiotics.

Investigation of the importance of the inoculum for penicillin sensitivity by the method described on p. 45 was carried out using King's serotype C and bacteraemia strain 5233. The results obtained with serotype C are shown in Table 10 (p. 48). It was found that there was only a weak inoculum effect after growth in both broth and broth containing penicillin. The inoculum effect was even weaker for strain 5233.

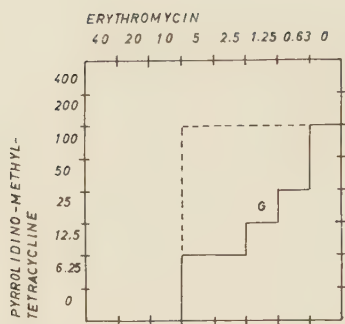


Fig. 7. A diagram depicting results obtained by a combination of erythromycin/pyrrolidion-methyl-tetracycline using method 3c (p. 44). The figures represent concentrations in $\mu\text{g/ml}$ of the two antibiotics. Growth appeared in the tubes below and to the right of the polygonal line. Tube G contains $1/8$ of the least inhibitory concentration of both antibiotics. Thus a potentating effect is present.

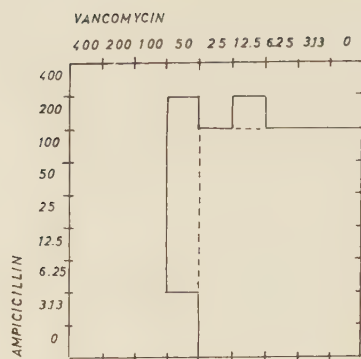


Fig. 8. A diagram depicting results obtained by a combination of vancomycin/ampicillin. Antagonism exists as growth appeared in several tubes placed in zones where the lowest inhibitory concentration of the one antibiotic was exceeded.

The method used is described on p. 44.

Table 10

Penicillin $\mu\text{g/ml}$	Culture grown in broth				Culture grown in broth containing penicillin $10 \mu\text{g/ml}$			
	undiluted	10^{-2}	10^{-4}	10^{-6}	undiluted	10^{-2}	10^{-4}	10^{-6}
125.....	0	0	0	0	0	0	0	0
62.5.....	0	0	0	0	+	0	0	0
31.75....	25	0	0	0	+	0	0	0
15.87....	++	580	26	4	++	++	++	574
7.94.....	++	++	++	59	++	++	++	+
3.97.....	++	++	++	85	++	++	++	+
0.....	++	++	++	100	++	++	++	+

Colony counts of *Flavobacterium meningosepticum* (King's serological type C) on agar plates containing penicillin. Method see p. 45.

+ too numerous to count, ++ confluent growth.

The sensitivity pattern is unusual for a gram-negative rod. It more closely resembles that of a gram-positive bacterium, as the micro-organism is most sensitive to erythromycin, novobiocin and vancomycin. It has been found to be extremely resistant to polymyxin B and sulphadimidine, and there is also marked resistance to streptomycin. There is somewhat less resistance to chloramphenicol, tetracycline and ampicillin.

King (1959) and Plotkin & McKittrick (1966) have obtained similar results. Other workers have also found sensitivity to streptomycin and tetracycline (Vandepitte et al.

1958; Sugathadasa & Arseculeratne 1963; Vandepitte et al. 1965). Watson et al. (1966) found that serotype F was sensitive to chloramphenicol, and this type has also been found by both the methods employed in this investigation to be more sensitive to chloramphenicol than the others.

Two of the strains isolated from the environment in Odense County and City Hospital (L 5 and T 138) were found on repeated investigations to be more resistant to erythromycin than the remainder.

The clinical effect of an antibiotic depends, in addition to its inhibitory effect on the micro-organism, on the concentration which can be achieved in the tissues. The usual division into the sensitivity groups + + +, + +, + and 0 is based on this. Thomsen (1967) has given the IC 50 values corresponding to these sensitivity groups for a number of antibiotics. If Thomsen's values are applied to the IC 50 values which have been found by the tube dilution method in the present study, then *Flavobacterium meningosepticum* is found to have the following resistance pattern: novobiocin + +, erythromycin, vancomycin and fucidin +, penicillin, tetracycline, ampicillin, chloramphenicol, streptomycin, sulphadimidine and polymyxin B 0.

In a number of cases the zones of inhibition obtained by the tablet method used here were thus too large (Table 7, p 46). This has been emphasized by Thomsen (1967) as characteristic when the tablet method is used for investigation of slowly growing bacteria, to which group *Flavobacterium meningosepticum* belongs (p. 37).

CONCLUSION

Flavobacterium meningosepticum has been found to be extremely resistant to antibiotics, and it must be expected that infections with this micro-organism will be difficult to treat by means of antibiotics. On combining antibiotics in vitro the increase in effect was so modest that it is not to be expected that a combination of antibiotics will be of any therapeutic importance.

The sensitivity of *Flavobacterium meningosepticum* to antibiotics at different temperatures

Flavobacterium meningosepticum has been found to have a very low sensitivity to antibiotics. There was therefore reason to investigate whether the sensitivity of the bacterium to antibiotics could be increased when the temperature was raised, as has been described in a number of other bacteria such as those mentioned in Chapter 1 (p. 15).

In this chapter a description is given of the sensitivity of *Flavobacterium meningosepticum* to a number of antibiotics in the temperature range 22°–39°C.

METHODS

1. The sensitivity at the temperatures 35°, 37°, 38° and 39°C was investigated for the following antibiotics: penicillin, vancomycin, ampicillin, erythromycin, novobiocin, fucidin, chloramphenicol, pyrrolidino-methyltetracycline (Reverin®), streptomycin and sulphadimidine.

A number of two-fold serial dilutions of the antibiotics dissolved in broth were prepared, with 0.5 ml in each tube. The tubes were inoculated with a platinum loop from a ten-fold dilution of an overnight broth culture incubated at 35°C. One duplicate series was incubated at each of the temperatures mentioned above. The number of tubes with growth after 2 days was used to determine the log₂ IC 50.

2. Equal amounts of erythromycin were added to four tubes containing 10 ml broth. The tubes were then inoculated with 0.1 ml of an overnight broth culture grown at 35°C. The tubes were incubated at room temperature (22°C) and at 30°, 37° and 39°C. At intervals the number of bacteria in the four cultures was determined by counting on blood agar plates as described earlier (p. 36). A simultaneous control experiment was carried out, in which the bacteria in a broth culture which did not contain erythromycin were counted in a similar manner.

The experiment was carried out using 20 and 25 µg erythromycin per ml.

Strains employed

The six serologically different strains (King A–F) described on p. 29 were investigated by method 1. King's serological type C was investigated by method 2.

RESULTS

Method 1

Table 11 and Table 12 show the results of the tests using chloramphenicol and penicillin. It is apparent that with chloramphenicol there was a slight fall in $\log_2$ IC 50 with increasing temperature, whilst with penicillin there would seem to be more of an increase.

Table 11

	King A		King B		King C		King D		King E		King F	
35°C.....	5.5	6.5	6.0	5.5	6.5	6.5	5.5	5.5	5.5	6.0	3.5	3.5
37°C.....	6.5		5.5		6.5		5.5		5.5		2.5	
38°C.....	4.5	5.5	5.5	5.0	5.5	6.5	4.5	5.5	4.5	4.5	1.5	1.5
39°C.....	3.5	4.5	5.0	4.5	5.5	5.5	3.5	5.0	4.5	3.5	1.5	1.5

The sensitivity to chloramphenicol expressed as $\log_2$ IC50 for six strains of *Flavobacterium meningosepticum* (King A-F) at temperatures 35°, 37°, 38° and 39°C. Two tests per strain. The sensitivity increased with temperature. The method used is described on p. 50.

Table 12

	King A		King B		King C		King D		King E		King F	
35°C.....	6.0	4.5	5.5	4.5	5.5	4.0	6.0	6.0	5.5	4.5	7.0	5.5
37°C.....	4.5		5.5		4.5		6.0		5.5		6.5	
38°C.....	6.5	5.5	7.0	5.5	5.5	4.5	7.0	6.5	6.0	5.5	7.5	6.5
39°C.....	6.5	5.5	6.5	6.5	5.5	4.5	7.5	5.5	5.5	5.0	6.0	4.5

The sensitivity to penicillin expressed as $\log_2$ IC50 for six strains of *Flavobacterium meningosepticum* (King A-F) at temperatures 35°, 37°, 38° and 39°C. The sensitivity was unchanged or fell with increasing temperature. The method used is described on p. 50.

Table 13 (p. 52) shows the mean values for the fall in $\log_2$ IC 50 for the six strains investigated at all the temperature intervals used, and for all ten of the antibiotics employed. Those falls in titre in which $P < 0.01$ are indicated by **, those in which $P < 0.05$ are indicated by *.

A fall in $\log_2$ IC 50, indicative of an increase in effect with increasing temperature, was found with the following antibiotics: erythromycin, novobiocin, fucidin, tetracycline, chloramphenicol, streptomycin and sulphadimidine. The fall was most marked with erythromycin.

An increase, or no change, in $\log_2$ IC 50, indicative of a diminished or unchanged effect, was found with penicillin, ampicillin and vancomycin. However, in the temperature interval 38°/39° it was found that ampicillin and vancomycin were more effective.

Table 13

	Temperature interval °C					
	35°/37°	35°/38°	35°/39°	37°/38°	37°/39°	38°/39°
Penicillin	-0.58*	-0.75**	-0.38	-0.25	0.17	0.38
Ampicillin	-0.67**	-0.88**	-0.54**	-0.25	-0.08	0.33**
Vancomycin	-0.17	0.42	0.83	0.25	0.83*	0.42*
Erythromycin	2.00**	2.58**	4.25**	0.42		1.58**
Novobiocin	0.58	1.08**	2.38**	0.75*	2.17**	1.29**
Fucidin	0.17	1.21**	2.38**	1.00**	2.08**	1.17**
Tetracycline	0.83**	1.58**	2.54**	0.83**	1.50**	0.96**
Chloramphenicol	0.25	0.96**	1.50**	0.58*	1.25**	0.54**
Streptomycin	0.58*	1.08**	2.67**	0.67*	2.33**	1.58**
Sulphadimidine	1.17**	2.29**	3.42**	0.83**	2.08**	1.13**

The mean fall of $\log_2$ IC50 for six strains of *Flavobacterium meningosepticum* studied at six temperature intervals with ten antibiotics using the method described p. 50. ** indicates that the mean fall is significant at the 1 per cent level, * that it is significant at the 5 per cent level (the t-test was used).

Method 2 (p. 50)

The results obtained with 20 μg erythromycin per ml is shown graphically in Fig. 9 and that obtained with 25 $\mu\text{g}/\text{ml}$ in Fig. 10. Time in hours is shown on the abscissa, and the logarithm of the number of bacteria in 0.1 ml on the ordinate.

Using 20 μg erythromycin per ml there was a more rapid fall in the bacterial count

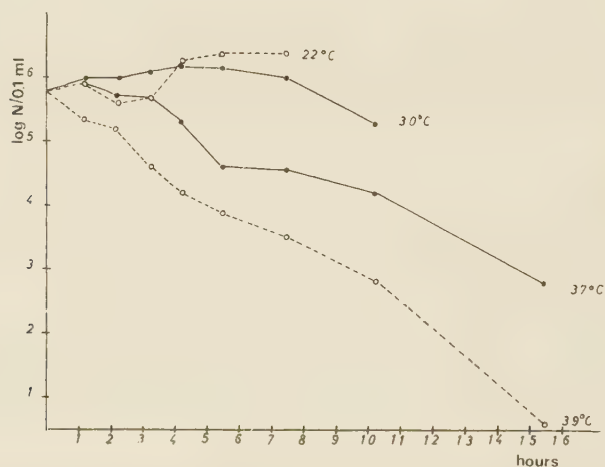


Fig. 9. The logarithm of the number of bacteria per 0.1 ml in broth cultures containing 20 $\mu\text{g}/\text{ml}$ of erythromycin as a function of the time at the temperatures 22°, 30°, 37° and 39° C (method 2, p. 50). (*Flavobacterium meningosepticum* King's serological type C).

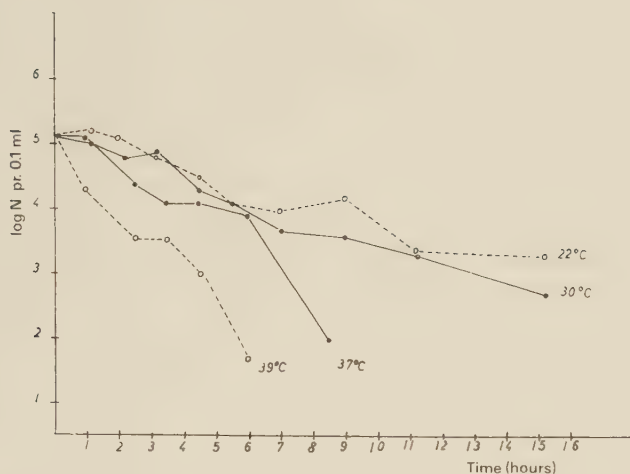


Fig. 10. Experiment as in Fig. 9 except that the broth contained 25 $\mu\text{g/ml}$ of erythromycin.

at 39° than at 37°C, whilst there was little change at 22° and 30°C. With 25 $\mu\text{g/ml}$ the counts also fell at the two last temperatures and the fall at 37° and 39°C was more rapid than had been the case at a concentration of 20 μg erythromycin per ml.

Table 14 contains the results of the bacterial counts in the broth cultures to which no erythromycin was added, when these were incubated at the same four temperatures. The experiment was carried out simultaneous with, and using the same culture as, the experiment with 20 μg erythromycin per ml.

Table 14

Hours from start of the experiment	The logarithm of the number of bacteria per 0.1 ml at the following temperatures			
	22°C	30°C	37°C	39°C
0.00.....	5.77	5.77	5.77	5.77
1.25.....	4.99	6.09	6.20	6.45
2.25.....	5.02	6.79	6.89	7.00
3.25.....	5.89	7.19	7.44	7.48
4.25.....	6.49	7.16	6.78	6.96
5.50.....	6.91	7.35	7.09	7.05
7.50.....	7.23	7.90	7.60	7.78
10.25.....	7.22	7.95	8.48	7.85
15.50.....	7.74	8.28	7.59	7.54
24.50.....	7.85	8.17	7.95	8.00

Flavobacterium meningosepticum (King's serological type C).

The logarithm of the number of bacteria per 0.1 ml when the bacteria was grown in broth at the temperatures 22°, 30°, 37° and 39°C. The experiment was carried out simultaneously with, and using the same culture as that used for the experiment with broth + 20 $\mu\text{g/ml}$ of erythromycin (Fig. 9).

..... indicates end of the exponential phase.

The values at 30°, 37° and 39°C were almost the same. At 22°C the values were somewhat smaller during the exponential phase, which lasted rather longer, but the final bacterial count was the same as that obtained at the other three temperatures. Slower growth at 22°C than at the other three temperatures used here was also found by the methods described in Chapter 5 (p. 36) (Olsen 1966).

These investigations have demonstrated that the effect of penicillin, ampicillin and vancomycin is independent of temperature, or else deteriorates with increasing temperature, whilst the effect of a number of other antibiotics improves with increasing temperature. It has furthermore been shown that this is not due to the fact that the bacterial count was different at the various temperatures investigated (Table 14, p. 53).

It is not possible to give any definitive explanation of the increasing effect of certain antibiotics with increasing temperature. The effect does not follow Arrhenius' law (p. 21), at all events not within the entire range of the temperatures which have been studied. Where erythromycin is concerned, no difference in effect has been demonstrated in the interval 22°/30°C, whilst there is an obvious difference in the interval 37°/39°C. This is not in accordance with Arrhenius' law.

Several of the antibiotics which in the present investigation have been demonstrated to have greater effect with increase in temperature also have an inhibitory action on protein synthesis (Hahn 1966; Godtfredsen 1967). A second common feature of these antibiotics is that all except streptomycin are bacteriostatic.

Treatment of *Flavobacterium meningosepticum* infections

As described in Chapter 5, the sensitivity of *Flavobacterium meningosepticum* to antibiotics is weak, even when the antibiotics are combined. It would not be possible to obtain therapeutic concentrations of any of the antibiotics shown in Table 8 (p. 47) in the cerebrospinal fluid unless they were administered intrathecally.

This low sensitivity to antibiotics, in combination with the reduced resistance in premature infants, provides an explanation for the high mortality rate in the meningitis in premature infants caused by *Flavobacterium meningosepticum*.

Almost every available antibiotic has been tried in the treatment of these infants. Because of the rarity of the disease no author has much experience with such cases but a few of them consider intrathecal vancomycin to be the most promising line of treatment (George et al. 1961; Plotkin & McKittrick 1966). In one case intraventricular erythromycin led to the sterilization of the cerebrospinal fluid after 4 days' treatment, with a concurrent improvement in the clinical state (Plotkin & McKittrick 1966). One case of bacteraemia and pneumonia, in which there were no signs from the central nervous system, recovered without sequelae on intramuscular erythromycin (George et al. 1961). In cases of meningitis extrathecal administration of erythromycin has been tried on many occasions but, as was to be expected, without clinical effect. In one case which had been treated with 250 mg erythromycin orally undiluted cerebrospinal fluid was found to have only a weak bacteriostatic action on *Flavobacterium meningosepticum* (George et al. 1961).

All workers have found the bacterium totally resistant to penicillin using the agar diffusion method. However, as may be seen from Table 8 (p. 47), *Flavobacterium meningosepticum* is more sensitive to penicillin than to those antibiotics which are usually effective against gram-negative rods. Perhaps intensive penicillin therapy is worth considering, although it might seem optimistic to expect it to be effective in consideration of the IC 50, which has been found in this study to be 24–95 µg/ml. Thanks to the atoxicity of penicillin it is nonetheless theoretically possible to achieve the necessary serum concentrations, which are assessed as 100–500 µg/ml. If the water-phase in new-born infants is taken as 75%, it would be possible to obtain 500 µg/ml by using a dose of 600,000 i.u./kg. Intrathecal injections of penicillin must be given concurrently.

However, intrathecal administration of penicillin is not without danger. Large doses have been followed by sudden death, and it is stated that for adults the dosage

should not exceed 20,000 i.u. by this route (Garrod 1964). There are also a number of publications which state that high concentrations of penicillin in the cerebrospinal fluid may give rise to convulsions (Weinstein et al. 1953; Dobell et al. 1966; Lerner et al. 1967). However, Weinstein et al. (1953) state that there are no risks associated with the administration of 30,000 i.u. twice daily to adults. This dosage would give a cerebrospinal fluid concentration of about 200 i.u./ml, which is sufficient to have a bacteriocidal effect on *Flavobacterium meningosepticum*. As the volume of cerebrospinal fluid in premature infants is in the range of 10–30 ml (Otila 1948), the permissible dosage would thus be 2000–6000 i.u. twice daily. The high serum concentrations which are reached by the extrathecal administration of the doses mentioned above must mean that the penicillin which is given intrathecally is excreted more slowly.

It has been mentioned in Chapter 7 that the sensitivity of *Flavobacterium meningosepticum* to a number of antibiotics increased with increasing temperature. However, the increase in the efficacy of the antibiotics was negligible at temperatures below 39°C. At 40°C the antibiotic effect will be supplemented by a marked inhibition of the growth of the bacteria (Olsen 1966). The chance of a satisfactory result of treatment will therefore be further increased in patients with fever. If the patient does not develop fever, then artificial hyperthermia is a line of treatment which should be contemplated. Even the thermoresistant variants are more sensitive to temperature than the majority of pathological bacteria, as may be seen from Table 1 (p. 18).

In infections with the thermolabile strain in the adult patients described in Chapter 3, the cases of bacteraemia gave rise to no therapeutic problems. As the patients showed high temperatures this is not surprising, as this strain is even more thermolabile than those bacteria of which it has been found that infections can be successfully treated with hyperthermia (group A, Table 1, p. 18). Had these patients not developed fever, then it might have been necessary to institute antibiotic therapy, and presumably erythromycin or vancomycin would have been the antibiotic of choice.

All the strains from meningitis patients which have been studied have been found to be thermoresistant and it is therefore not known whether meningitis can be caused by a thermolabile strain. Any strain which is isolated from a patient with meningitis should be investigated for temperature sensitivity, as because of the limited efficacy of the antibiotics hyperthermia therapy could be expected to be a valuable supplement to the therapeutic regimen if the strain were thermolabile.

CHAPTER 9

Epidemiological studies

This chapter contains a description of the investigations which have been carried out in order to clarify the epidemiological circumstances associated with the hospital endemic of *Flavobacterium meningosepticum* infections in the Department of Thoracic Surgery, Odense County and City Hospital, which has been described in Chapter 3.

Bacteriological technique

1. Agar plates containing 10% horse blood with 50 i.u. penicillin and 40 µg polymyxin per ml (slightly modified from the method given by King 1959) were used to isolate *Flavobacterium meningosepticum*. The plates were incubated at 35°C for 2 days.

2. The following technique was found useful for the investigation of water: a sufficient quantity of the substrate described below was added to the water to give an approximately 50 times dilution of the substrate:

Extract broth concentrated 10 times

Glucose 1%

Polymyxin 1 mg/ml

Penicillin 2000 i.u./ml

Incubation was at 30°C for 2 days.

However, the investigations described earlier in this monograph (Table 8, p. 47) suggest that this concentration of penicillin was too high.

RESULTS

A more detailed description of the results has been given in an earlier report (Olsen 1967b), and therefore only the most important features will be described here.

1. *Investigations of human carriers*

It was not possible to demonstrate human carriers by any of the following methods:

a. Swabs were taken from the nose and throat of 325 patients who underwent operation in the Department of Thoracic Surgery in the period 5/8 1964–5/1 1965. They were taken shortly after operation and 24 hours later. In particular, no *Flavobacterium meningosepticum* could be demonstrated in the 3 patients who developed bacteraemia during this period (Table 2, p. 24).

b. *Investigation of sputum.* All sputum samples received by the Regional Laboratory

of Statens Seruminstitut in Odense during the period 1/1 1965–1/4 1965 were routinely cultured for *Flavobacterium meningosepticum*. A total of 463 samples were studied in this manner.

c. Swabs were taken from the nose and throat of all the theatre staff in the Department of Thoracic Surgery. A total of 18 persons were involved, and all were also investigated for agglutinating antibodies in the serum. No such antibodies were found.

2. *Investigations of environment and instruments*

The investigations were carried out in all the five surgical units of the hospital, and in the wards of the Department of Thoracic Surgery. A total of 269 samples were studied.

Strains identical or closely related to those from the patients with bacteraemia were rapidly discovered in the environment. The strains were isolated in particular from damp areas such as wash-basins, suction apparatus and the humidifiers attached to the operating theatres. Three different strains were demonstrated:

a. Strains which were biochemically identical to those isolated from the patients with bacteraemia.

b. Strains differing from a. in being less sensitive to erythromycin.

c. Strains differing from a. in forming a yellow pigment and not producing acid from ethyl alcohol.

Strains identical to the bacteraemia strains were found in the operating theatre and wards of the Department of Thoracic Surgery, and in the operating theatres of the Departments of General Surgery, Obstetrics and Gynaecology, and Neurosurgery.

All three strains were isolated on many occasions from the humidifiers attached to the operating theatres. This was also the case after the endemic of bacteraemia had ended. Two years after the last case of bacteraemia 12 samples were taken over a period of 6 weeks from the water in the humidifier attached to the operating theatre of the Department of Thoracic Surgery. *Flavobacterium meningosepticum* was demonstrated in all 12 samples. A total of 13 strains were isolated, 8 of which were identical to the bacteraemia strains, whilst 5 belonged to the group with the greater resistance to erythromycin.

3. *Investigation of the air*

These investigations were made using Bourdillon's apparatus, in which air is sucked through 1–4 narrow slits and then hits a rotating plate containing medium (Bourdillon et al. 1948). The penicillin-polymyxin plate described on p. 57 was used as medium.

On 22/2 1965 between 9 a.m. and 9 p.m. a total of 27 samplings were made, equal numbers of samples being taken from the two operating theatres and the staff's scrub-room in the Department of Thoracic Surgery. A total of 38.07 m³ of air was investigated, and the 27 medium plates grew 153 colonies of *Flavobacterium meningosepticum*, i.e. 4.01/m³.

During the entire 12-hour period nine medium plates were left in the same rooms for spontaneous settling. This corresponded to an area of 1.61 m² for one hour. During the 12 hours a total of six bacteria settled on the nine plates.

The bacteria found were the pigment-forming variant which did not produce acid from ethyl alcohol. However, on previous occasions sedimentation had also revealed the erythromycin-resistant variant in the air.

Two years later repetition of the investigation revealed no *Flavobacterium meningosepticum* in a total of 145 m³ air, despite the fact that, as already mentioned, it was still present in the humidifiers. This could perhaps be due to the introduction of an anti-septic cleaning agent during the intervening period.

From the results of the air studies it is possible to calculate the settling velocity and size of the particles carrying *Flavobacterium meningosepticum* from the following equation:

$$v = \frac{S}{N} = 0.1153 \text{ d}^2$$

where v is the settling velocity in m/h, S the number of bacteria settling/m²/h, N the number of bacteria/m³ in the air, and d the particle diameter in μ (see, e.g. Siboni 1960).

However, in this study the calculation would be associated with a high degree of uncertainty, as only six bacteria settled. In addition to the calculations an attempt has therefore been made to obtain an impression of the values by comparison with a larger, corresponding, material from a study of *Staphylococcus aureus* (Siboni 1960).

In that study a total of 387 staphylococci were found in 124.08 m³ air, whilst on the settle plates there were 138 colonies on 2.46 m² in one hour. In order to render the two studies comparable it was calculated how many staphylococci would have been found in the same amount of air and the same settling area as that used in the study of *Flavobacterium meningosepticum*. These values are shown in Table 15, which also shows the calculated settling velocity and diameter of the bacteria-carrying particles for the two types of bacteria.

Table 15

	Number of bacteria in 38.07 ³ of air	Number of settled bacteria on 1.61 m ² /h	Settling velocity m/h	Diameter of the particle (μ)
<i>Flavobacterium meningosepti-</i> <i>cum</i>	153	6	0.92	2.83
<i>Staphylococcus aureus</i>	119	91	17.96	12.52

Settling velocity and diameter af airborne particles of *Flavobacterium meningosepticum* compared with *Staphylococcus aureus*.

It is apparent from the table that the air content of *Flavobacterium meningosepticum* was higher than that of *Staphylococcus aureus*, and it would be anticipated that at least the same number would settle if the size of the bacteria-carrying particles were the same for the two types of bacteria. However, it was found that far fewer *Flavobacterium meningosepticum* bacteria settled $\left(\chi^2 = \frac{(91 - 6)^2}{6} = 79.40, f = 1\right)$. This must

be due to a lower settling velocity for the particles carrying *Flavobacterium meningosepticum* than those carrying *Staphylococcus aureus*. The particle size calculated for the particles carrying *Flavobacterium meningosepticum* corresponds to that of droplet nuclei.

4. Investigation of drugs

Two drops of the concentrated substrate containing antibiotics (described on p. 57) were added to the vials of injection preparations in use in the Anaesthetic Department. After incubation at 30°C for 2 days was inoculated onto blood agar plates and Conradi Drigalski plates.

Using this technique it was possible to demonstrate *Flavobacterium meningosepticum* in both commercial preparations and preparations made up in local pharmacy. The contaminated preparations were found in the Departments of Thoracic Surgery and Orthopaedics.

All the preparations in use in the Department of Anaesthetics were then withdrawn. Unused preparations with the same batch number as those which were contaminated were found to be sterile, and no *Flavobacterium meningosepticum* could be demonstrated in 209 vials which were taken into use after the withdrawal of the contaminated vials. The investigations which were carried out exclude the possibility of the preparations having been contaminated at source.

The investigation revealed that *Flavobacterium meningosepticum* was widely distributed in Odense County and City Hospital. Nonetheless no healthy carriers were demonstrated and only patients who had received intravenous anaesthetics became ill. This must be considered as an expression of the fact that *Flavobacterium meningosepticum* is a micro-organism of low pathogenicity.

The micro-organism has seldom been isolated from adults. Only 4 of King's 68 strains were isolated from adults (King 1965). There is a report of one case of meningitis in an adult in which the bacterium was isolated from the cerebrospinal fluid (King 1959). The patient was given intensive antibiotic therapy and recovered. The present material includes one patient (no. 9, p. 23) in whom true infection was not demonstrated, but where *Flavobacterium meningosepticum* was found in the sputum. The patient at that time suffered from uraemia and therefore most probably had reduced resistance.

By contrast, *Flavobacterium meningosepticum* has been demonstrated many times in the nose and throat of healthy new-born infants during meningitis endemics

(Brody et al. 1958; Cabrera & Davis 1961). During a hospital endemic in Israel Seligmann et al. (1963) more commonly found healthy carriers among premature than among mature infants.

On the basis of all these observations it would seem justified to conclude that *Flavobacterium meningosepticum* thrives poorly in adult individuals. In the new-born it may be found in the nose and throat, and in the premature it may cause severe meningitis.

The source and route of the infection in the Department of Thoracic Surgery must be considered to have been elucidated, after the demonstration of *Flavobacterium meningosepticum* in the drugs for intravenous administration. Moreover no new cases have arisen since the named precaution was adopted. The drugs may have been contaminated from the environment, where the bacteria were widely disseminated. The contamination of the vials was probably maintained by the transfer of the bacteria from one vial to another. After the constant contamination of drugs for one year it would have been expected that there would have been a greater number of infections than those diagnosed, which comprised only 1–2% of the total number of patients who underwent operations. This presumably means that a number of patients have had the bacterium injected without becoming ill. This might also explain the finding that none of the patients in the Orthopaedic Department developed bacteraemia, despite the fact that *Flavobacterium meningosepticum* was also found in the drugs for intravenous injection in that department.

In two cases it has proved possible to demonstrate a possible source of infection during hospital endemics of meningitis in premature infants caused by *Flavobacterium meningosepticum*. Cabrera & Davis (1961) demonstrated the micro-organism in a wash-basin in the ward, and the endemic ended after the wash-basin had been replaced. Plotkin & McKittrick (1966) demonstrated the bacterium in a saline solution which had been used to irrigate the eyes. In other cases no sources of infection was found (Brody et al. 1958; Vandepitte et al. 1958; Seligmann et al. 1963; Watson et al. 1966).

CHAPTER 10

Strains resembling *Flavobacterium meningosepticum* isolated outside the hospital and as random findings in the routine investigation of specimens from patients

This chapter contains a description of investigations which were carried out to isolate *Flavobacterium meningosepticum* from tap water, surface water (Odense River) and cultivated soil.

All the strains isolated during the investigation which resembled *Flavobacterium meningosepticum* in that they were aerobic, oxidase-positive and non-motile are described in the following. In addition a description is given of strains with these properties which were isolated on routine investigation of specimens from patients who manifested no clinical signs of infection with *Flavobacterium meningosepticum*.

MEDIA AND METHODS

In the isolation of *Flavobacterium meningosepticum* advantage was taken of the marked resistance to antibiotics displayed by the micro-organism (Table 8, p. 47) which meant that antibiotics could be added to the media employed without this inhibiting growth. However, the patient specimens were investigated without the addition of antibiotics, being spread on 5% blood agar plates and Conradi Drigalski plates. The following media containing antibiotics were used:

- A. 10% horse blood agar with 20 i.u. penicillin + 40 µg polymyxin B per ml.
- B. 10% horse blood agar with 10 i.u. penicillin + 40 µg streptomycin + 40 µg polymyxin B per ml.
- C. Conradi Drigalski plates with 40 µg polymyxin B per ml.
- D. Agar plates with 10 i.u. penicillin + 40 µg streptomycin + 40 µg polymyxin B per ml, with 1% mannitol and bromthymol blue as indicator.
- E. The following substrate was added to the water samples in an amount such that it was diluted 50 times:

Broth concentrated 10 times
Glucose 1%
Polymyxin B 1 mg/ml
Penicillin 1000 i.u./ml

The water samples were then incubated at 30°C for 2 days.

The media described were incubated at 35°C for 2 days.

By inoculating plates with and without the addition of antibiotics with known

strains of *Flavobacterium meningosepticum*, followed by counting of the colonies, it was ascertained that the antibiotics which had been added did not inhibit growth.

Investigation of tap water

1. The tap was flamed and allowed to run for 10 minutes. A sample of 1000 ml was collected and filtered through a membrane filter (Sartorius membrane filter 11406–50 mm, Germany). The filters were placed on medium A. A total of 15 litres of water was investigated.

2. The same technique as 1., but the filters were placed on medium B. 23 litres was investigated.

3. 250 ml water was collected in a sterile glass flask. 5 ml of medium E was added and the flasks were incubated for 2 days. After this 0.1 ml was inoculated onto a 5% blood agar plate, a Conradi Drigalski plate and also a blood plate for sensitivity determinations using the Sensitabs® described on p. 42, on the supposition that it would be possible to facilitate the isolation on the basis of the characteristic sensitivity pattern of *Flavobacterium meningosepticum*.

Investigation of river water

1. 0.1 ml water was inoculated on medium C and a resistance plate as described above. A total of 3 ml was investigated.

2. 10 ml river water was filtered through a membrane filter, which was then placed on medium B. A total of 90 ml was investigated.

Investigation of soil samples

1. Soil from a sample of about 1 cm³ was spread with a cotton swab on medium C. 5 ml water and 0.1 ml medium E were added to the remainder of the sample. After incubation for 2 days the culture was spread with a platinum loop on blood agar plates, Conradi Drigalski plates, and plates for the determination of antibiotic sensitivity. A total of 47 samples was investigated.

2. Samples were spread with a swab on media B and C. A total of 32 samples was investigated.

Pure cultures of all the strains isolated by the methods described above were made on Conradi Drigalski plates and investigated for the following properties by the methods described on p. 28: aerobic or anaerobic growth, motility, oxidase production, indole production, nitrate reduction, liquefaction of gelatine, splitting of urea and ability to utilize citrate. Furthermore their sensitivity to the following eight antibiotics was tested with Sensitabs as described on p. 42: sulphamethizole, penicillin, tetracycline, streptomycin, chloramphenicol, erythromycin, colimycin and kanamycin.

All aerobic, non-motile, oxidase-positive strains were furthermore investigated for acid production from the 21 carbohydrates named on p. 30.

3. On the basis of the experience which had been gained in the course of the

investigation a further 32 soil samples were studied by the following method, which differed from those described above in that a number of strains which differed from *Flavobacterium meningosepticum* were primarily excluded:

The samples of soil were spread with a swab on media B and D. After pure cultivation on Conradi Drigalski plates all colonies were investigated for growth in semi-solid agar and for production of acid from mannitol and salicin. Those strains which produced acid from mannitol but not from salicin, and which were furthermore aerobic, oxidase-positive and non-motile were tested for indole production, liquefaction of gelatine, nitrate reduction, urease production and ability to utilize citrate. Strains which proved to be indole-negative, and which did not liquefy gelatine or reduce nitrate were rejected, whilst the remainder were investigated for acid production from the 21 carbohydrates already named, and for sensitivity to the eight antibiotics.

RESULTS

Table 16 shows the properties of all the aerobic, oxidase-positive, non-motile strains isolated from patient specimens, soil, river and tap water, and also some strains isolated from the surroundings of the Odense County and City Hospital. There were a total of 291 strains, 134 of which were different. Those strains which

Isolated from	Number of strains	Group no.	Aerobic oxidase positive					Isolated from patient specimens, hospital surroundings					
			Indole	Nitrate reduction	Gelatin	Urea	Citrate	Sulphamethizole	Penicillin	Tetracycline	Streptomycin	Chloramphenicol	Erythromycin
Patient specimens, hospital surroundings, river water, soil	21	1	+	—	+	+	+	0	0	+	+	+	++
Hospital surroundings..	19	2	+	—	+	+	+	0	0	+	+	+	+
Patient specimens, soil..	18	3	+	—	+	+	+	0	0	+	+	+	++
Patient specimens, hospital surroundings, river water, soil	13	4	+	—	+	+	+	0	0	+	+	+	++
Soil.....	1	5	+	—	+	+	+	0	0	+	+	+	++
Soil.....	1	6	+	—	+	+	+	0	0	+	++	++	++
Soil.....	1	7	+	—	+	+	+	0	0	+	+	+	++

resembled *Flavobacterium meningosepticum* most are shown first in the table. Groups 1–55 thus comprise strains possessing three properties characteristic of *Flavobacterium meningosepticum*, i.e. indole production, liquefaction of gelatine and lack of ability to reduce nitrate.

Flavobacterium meningosepticum in soil, river water and patient specimens.

Table 17 (p. 72) shows the numbers of aerobic, oxidase-positive, non-motile strains which were isolated from soil, river water, patient specimens and tap water. The table also shows the number of strains isolated from these four sources which had properties identical to those of *Flavobacterium meningosepticum*, and also those which differed only in that they did not produce acid from lactose and ethyl alcohol. This may be considered as a minor deviation as, as mentioned on p. 30, some of the strains of *Flavobacterium meningosepticum* did not produce acid from lactose until late, and one strain (T 150) did not produce acid from ethyl alcohol. Table 17 does not include those strains isolated by the selective method described on pp. 63–64 (method 3). These are shown separately in Table 18 (p. 73).

It is apparent from Table 17 that strains identical to *Flavobacterium meningosepticum* were isolated from soil, river water and patient specimens, but not from tap water.

The patient specimens included urine, blood, sputum and cerebrospinal fluid.

non-motile strains.
findings, tap water, river water and soil.
k reaction

Colimycin	Kanamycin	Glucose	Lævulose	Maltose	Lactose	Trehalose	Mannitol	Glycerol	Ethanol	Arabinose	Xylose	Rhamnose	Galactose	Sucrose	Raffinose	Starch	Inulin	Adonitol	Dulcitol	Sorbitol	Inositol	Salicin
0	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
0	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
0	+	+	+	+	—	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
0	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0	0	+	+	+	+	+	—	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
0	+	+	+	+	+	+	(+)	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0	+	+	+	+	—	+	(+)	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—

Table 16 (cont.)

Isolated from	Number of strains		Nitrate reduction				Sulphamethizole	Penicillin	Tetracycline	Streptomycin	Chloramphenicol		
	Group no.		Indole		Gelatine	Urea	Citrate,						
Hospital surroundings,													
river water, soil.	6	8	+	—	+	+	+	0	0	+	+	+	+
Soil.	1	9	+	—	+	+	+	0	0	+	+	+	+
Soil.	1	10	+	—	+	+	+	0	0	+	+	+	+
Soil.	5	11	+	—	+	+	+	0	0	+	+	+	+
Soil.	6	12	+	—	+	+	+	0	0	+	+	+	+
Soil.	6	13	+	—	+	+	+	0	0	+	+	+	+
Soil.	17	14	+	—	+	+	+	0	0	+	+	+	+
Soil.	1	15	+	—	+	+	+	0	0	+	+	+	+
Soil.	1	16	+	—	+	+	+	0	0	+	+	+	+
Soil.	10	17	+	—	+	+	+	0	0	+	+	+	+
Soil.	2	18	+	—	+	+	+	0	0	+	+	+	+
Soil.	1	19	+	—	+	+	+	0	0	+	+	+	+
Soil.	12	20	+	—	+	+	+	0	0	+	+	+	+
Soil.	1	21	+	—	+	+	+	0	0	+	+	+	+
Soil.	1	22	+	—	+	+	+	0	0	+	+	+	+
Soil.	1	23	+	—	+	+	—	0	+	+	+	+	+
Soil.	1	24	+	—	+	+	+	0	0	+	+	+	+
Soil.	1	25	+	—	+	+	—	0	0	+	+	+	+
Soil.	2	26	+	—	+	+	+	0	0	+	+	+	+
Soil.	1	27	+	—	+	—	—	0	0	+	+	+	+
Hospital surroundings .	1	28	+	—	+	—	—	0	0	+	++	+	++
Patient specimen	1	29	+	—	+	+	+	0	0	+	+	++	++
Hospital surroundings .	1	30	+	—	+	+	+	0	0	+	+	+	++
Soil.	2	31	+	—	+	+	+	0	0	+	+	+	++
Soil.	1	32	+	—	+	+	+	0	0	+	+	++	++
Soil.	1	33	+	—	+	+	+	0	0	+	+	+	++
Soil.	1	34	+	—	+	+	—	0	+	+	+	++	++
Hospital surroundings .	1	35	+	—	+	—	—	0	0	+++	+++	+++	++
Soil.	1	36	+	—	+	+	+	0	+	+	+	+	++
Patient specimen	1	37	+	—	+	+	+	+	0	+	0	++	++
Soil.	1	38	+	—	+	+	—	0	0	+	+	+++	++
Soil.	1	39	+	—	+	+	—	0	0	+	++	+	++
Patient specimen	1	40	+	—	+	+	+	0	0	+++	+++	+++	++
Soil.	1	41	+	—	+	+	+	0	0	+	+	+	++
Soil.	5	42	+	—	+	+	+	0	0	+	+	++	++
Soil.	1	43	+	—	+	+	+	0	0	+	++	+	++
Patient specimen	1	44	+	—	+	+	—	0	+	+++	+++	+++	++
River water.	1	45	+	—	+	+	+	0	0	++	+++	++	++
Patient specimens.	2	46	+	—	+	+	+	0	0	+++	+++	+++	++
Soil.	1	47	+	—	+	+	+	0	0	+	+	+	++
Soil.	1	48	+	—	+	+	—	0	0	+	+	+	++
Patient specimen	1	49	+	—	+	+	—	0	0	+	++	++	++

Table 16 (cont.)

Isolated from	Number of strains	Group no.	Indole	Nitrate reduction	Gelatine	Urea	Citrate	Sulphamethizole	Penicillin	Tetracycline	Streptomycin	Chloramphenicol
Patient specimen.....	1	50	+	-	+	+	-	0	+++	+++	+++	+
Tap water.....	1	51	+	-	+	-	+	0	+	+++	+++	+
River water.....	1	52	+	-	+	-	+	+	+	+++	+++	+
Tap water.....	1	53	+	-	+	-	-	0	0	+++	+++	+
Soil.....	1	54	+	-	+	+	+	0	0	++	++	+
Patient specimen.....	1	55	+	-	+	+	-	+	+	++	+++	+
River water.....	1	56	+	+++	+	+	+	+	0	+	++	+
River water.....	1	57	+	+++	+	+	+	0	0	+	+	+
Soil.....	1	58	+	+++	+	(+)	-	0	0	+	+	+
River water.....	1	59	+	-	-	+	+	0	0	+++	++	+
Soil.....	1	60	+	-	-	+	-	0	0	+	++	+
Soil.....	4	61	+	-	-	+	-	0	0	+	+	+
Patient specimen.....	1	62	+	-	-	-	+	0	+	+++	++	+
Patient specimen.....	1	63	+	-	-	-	-	+	+	+++	+++	+
River water.....	1	64	+	-	-	-	-	0	0	+++	+	+
River water.....	1	65	+	-	-	-	-	++	0	+++	+	+
Patient specimen.....	1	66	+	-	-	+	-	+++	++	+++	+++	+
Soil.....	1	67	+	+	+	+	+	0	0	+	+	+
River water.....	1	68	+	+	-	+	-	+++	0	0	++	+
River water.....	1	69	-	+	-	+	+	+++	0	+++	+++	+
River water.....	1	70	-	+	-	-	+	0	0	+++	+	+
River water.....	1	71	-	+	-	+	+	0	0	+	+	+
Soil.....	1	72	-	+	+	+	+	0	0	+++	+	+
River water.....	1	73	-	+	-	+	+	+++	0	+++	+++	+
River water.....	2	74	-	+	-	+	+	0	+++	+++	+++	-
Hospital surroundings..	1	75	-	-	+	+	+	0	0	+	+	+
River water.....	1	76	-	-	+	+	-	+	0	0	++	+
Soil.....	1	77	-	-	+	+	-	0	0	+	++	+
River water.....	1	78	-	-	+	+	+	0	0	0	+++	+
Soil.....	1	79	-	-	+	+	+	0	0	+	+	+
River water.....	1	80	-	-	+	+	-	0	0	+	+	+
River water.....	2	81	-	-	+	+	-	0	0	+	+	+
River water, patient specimen....	3	82	-	-	+	+	-	0	0	0	++	+
River water.....	1	83	-	-	+	+	-	0	+	0	+	+
River water.....	2	84	-	-	+	-	+	0	++	+++	+++	+
River water.....	5	85	-	-	+	+	-	0	+	+	++	+
River water.....	1	86	-	-	+	+	-	0	++	++	+	+
River water.....	3	87	-	-	+	+	-	0	+++	+++	++	+
River water.....	1	88	-	-	+	+	-	0	+++	+++	++	+
River water.....	1	89	-	-	+	+	+	0	0	+++	+	+
River water.....	1	90	-	-	+	+	-	0	0	++	+	+
Soil.....	1	91	-	-	+	+	-	+++	0	+++	+	+

Table 16 (cont.)

Isolated from	Number of strains	Group no.	Indol	Nitrate reduction	Gelatine	Urea	Citrate	Sulphamethizole	Penicillin	Tetracycline	Streptomycin	Chloramphenicol
River water.....	1	92	-	-	+	+	-	0	++	+++	++	+++
River water.....	1	93	-	-	+	-	-	++	+	+++	++	+++
Tap water.....	1	94	-	-	+	-	-	+	++	+++	+++	+
Soil.....	1	95	-	-	+	+	+	+++	0	+	++	+
River water.....	1	96	-	-	+	+	-	0	0	++	+	+++
River water.....	1	97	-	-	+	+	-	+++	+++	+++	+++	+
Patient specimen.....	1	98	-	-	+	+	-	0	+++	+++	+++	+
Patient specimen.....	1	99	-	-	+	+	+	0	0	+	0	+
Patient specimens.....	2	100	-	-	+	+	+	0	0	+++	0	+
Soil.....	1	101	-	-	+	+	-	0	0	+	0	+
River water.....	1	102	-	-	+	+	+	0	0	++	+++	+++
Soil.....	1	103	-	-	+	+	+	0	+	++	++	+++
Soil.....	1	104	-	-	+	+	+	+++	0	+++	+	+++
Soil, tap water.....	2	105	-	-	+	+	+	+	0	++	+	++
Soil.....	2	106	-	-	+	+	+	+	0	++	++	++
Soil.....	3	107	-	-	+	+	+	0	0	+	+	++
Soil.....	1	108	-	-	+	+	+	0	0	+	+	+
Soil.....	1	109	-	-	+	+	+	+	0	+	0	+
Soil.....	1	110	-	-	+	+	+	+++	0	+++	0	+
River water.....	1	111	-	-	-	+	-	0	0	+	+	+
River water.....	1	112	-	-	-	-	+	+	+	+++	+++	+
River water.....	2	113	-	-	-	+	+	++	0	+++	++	+++
River water.....	1	114	-	-	-	+	-	0	+++	+++	+++	+
River water.....	1	115	-	-	-	+	-	+	0	+++	0	++
River water.....	1	116	-	-	-	-	-	0	++	+++	+++	+
Soil.....	1	117	-	-	-	+	+	0	0	+	+	+
River water.....	1	118	-	-	-	+	+	0	0	+++	+++	+
River water.....	1	119	-	-	-	-	-	0	++	+++	++	+++
River water.....	1	120	-	-	-	+	-	0	0	+++	+++	+
River water.....	1	121	-	-	-	+	-	0	0	+++	+++	+
Soil.....	1	122	-	-	-	+	+	0	0	+++	++	+++
Soil.....	1	123	-	-	-	+	+	+++	0	+++	++	+++
Soil.....	3	124	-	-	-	+	+	0	0	+	+	++
Soil.....	6	125	-	-	-	+	-	0	0	+	+	++
Soil.....	1	126	-	-	-	+	+	0	0	+	+	++
Soil.....	1	127	-	-	-	+	-	0	+	+	++	++
River water.....	1	128	-	-	-	+	-	0	0	+++	+	+++
River water.....	1	129	-	-	-	+	-	0	0	+++	0	++
Patient specimen.....	1	130	-	-	-	-	-	0	+	+++	+++	+
Soil.....	1	131	-	-	-	+	+	0	0	++	+++	+++
Patient specimen.....	1	132	-	-	-	-	-	+++	+++	+++	+++	+
Tap water.....	1	133	-	-	-	-	+	+++	++	+++	0	+++
Patient specimen.....	1	134	-	-	-	+	-	+++	0	+++	+++	++

Colimycin	Kanamycin	Glucose	Lævulose	Maltose	Lactose	Trehalose	Mannitol	Glycerol	Ethanol	Arabinose	Xylose	Rhamnose	Galactose	Sucrose	Raffinose	Starch	Inulin	Adonitol	Dulcitol	Sorbitol	Inositol	Salicin
0	+++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	0	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
++	+++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
++	+++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
++	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
++	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
++	+++	+	+	-	-	-	-	+	+	+	+	-	+	-	-	+	-	-	-	-	-	-
++	+++	+	+	-	-	-	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-
0	0	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	-	-	-	-
0	0	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-	+	-	-	-	-	-
0	0	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	-	-	-	-	-
0	++	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	-	-	-	+
0	0	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	+
0	0	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	+
0	0	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	+
0	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
0	+++	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
0	+++	+	-	+	+	-	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-
0	+++	+	+	+	-	-	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-
0	+++	+	+	-	-	-	+	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-
0	+++	+	+	-	-	-	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-
0	+	+	+	+	+	+	-	+	-	+	+	-	+	+	+	-	+	-	-	-	-	-
0	+++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0	+++	+	+	(+)	-	-	-	+	+	+	+	-	+	-	-	-	-	+	+	(+)	-	-
0	+++	+	+	+	-	(+)	(+)	+	+	+	+	+	+	+	-	-	-	(+)	+	+	+	-
0	+++	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	-
0	0	+	+	+	+	+	-	-	-	+	+	-	+	+	+	(+)	+	-	-	-	-	+
0	+	+	+	+	+	+	-	-	-	+	+	-	+	+	+	-	+	-	-	-	-	+
0	0	+	+	+	+	+	-	-	-	+	+	-	+	+	+	-	+	-	-	-	-	+
0	0	+	+	+	+	+	-	-	-	+	+	+	+	+	+	(+)	+	-	-	-	-	+
+	+++	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
++	+++	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
++	0	+	-	-	-	-	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-
++	+++	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	-	+	+	+	-	-

Table 17

Source	total no. of strains	no. of differ- ent strains	Same prop- erties as Fl. mening.	Differ from Fl. mening. by not attacking		
				lactose	ethanol	lactose and ethanol
Soil						
(79 specimens examined)	78	50	1	2	2	1
River water						
(93 ml examined).....	60	46	3	0	2	1
Patient specimens						
(26 examined).....	26	21	1	3	2	0
Tap water						
(40 litres examined)....	5	5	0	0	0	0
Totals.....	169	122	5	5	6	2

Flavobacterium meningosepticum and other aerobic oxidase-positive non-motile strains isolated from four different sources.

Two of the 26 strains shown in Table 17 were isolated from specimens from patients in hospitals in Copenhagen. One of these was identical to *Flavobacterium meningosepticum* and was isolated from sputum. The remaining 24 strains were all isolated from specimens from Odense County and City Hospital. None of the patients showed any clinical evidence of infection with *Flavobacterium meningosepticum*, and the findings must presumably be due to either saprophytism or contamination.

As mentioned above, the bacterium was not isolated from tap water. The flora in tap water was dominated by aerobic, polar motile rods, but *Chromobacterium violaceum* was also isolated on a number of occasions. The polar motile rods differed from those found in patient specimens in being resistant to colimycin and in that they generally did not form pigment. Colimycin-resistant polar motile rods isolated from water have previously been described by Moffet & Williams (1967).

In river water the flora was dominated by *Proteus* and *Serratia*, and in soil by motile facultative anaerobic strains and a number of polar motile rods.

Aerobic, oxidase-positive, non-motile strains isolated from soil using the highly selective method 3 (p. 63)

The strains isolated by this method are included in Table 18 (p. 73). 75 strains, 17 of them different, were isolated from 32 samples. The group numbers shown in the left-hand column refer to those in Table 16. 72 of the strains had the same sensitivity pattern as *Flavobacterium meningosepticum*, the only groups which deviated being nos. 36, 38 and 54. As all these strains, from the method of isolation used, were indole-positive, liquefied gelatine and did not reduce nitrate, these 72 strains differed from *Flavobacterium meningosepticum* only in the carbohydrate reactions shown in Table 18. In no case was there acid production from lactose, but apart from this all the

Table 18

Group no. shown in table 16	No. of strains	Differ from <i>Flavobacterium meningosepticum</i> by	
		attacking	not attacking
3.....	13		lactose
9.....	1		lactose, ethanol
12.....	6	starch	lactose
13.....	4	sucrose	lactose
14.....	17	sucrose, starch	lactose
15.....	1		lactose, glycerol
17.....	10	starch	lactose, ethanol
18.....	2	sucrose	lactose, ethanol
19.....	1	arabinose	lactose, glycerol
20.....	12	sucrose, starch	lactose, ethanol
25.....	1	arabinose, xylose, sucrose	lactose
32.....	1	starch	lactose, ethanol, l��vulose
36.....	1	sucrose, galactose, raffinose	lactose, ethanol
38.....	1	sucrose, starch, xylose	lactose, ethanol
41.....	1	sucrose, starch	lactose, ethanol, mannitol
48.....	1	sucrose, starch	lactose, l��vulose, mannitol
54.....	1	sucrose, xylose	lactose, glycerol, mannitol
Total....	75		

Aerobic oxidase-positive non-motile strains isolated from soil using method 3 described on p. 63. 32 specimens were examined.

remaining divergencies were small, and were in the main due to differences in acid production from starch, sucrose and ethyl alcohol.

The last three strains (groups 41, 48 and 54) which on the agar plate containing mannitol were considered to produce acid from mannitol, were found in investigations using Hugh & Leifson's medium not to attack mannitol.

Aerobic, oxidase-positive, non-motile strains isolated by the other, less selective methods (without antibiotics or with the media A, B, C and E containing antibiotics (p. 62)

These strains comprise 117 of the 134 strains shown in Table 16 (pp. 64–71).

As several different methods had been used for isolation, the strains were not directly comparable. An attempt has been made to remedy this by dividing the strains into groups I, II and III, according to the antibiotics which were used in the isolation, as this was considered to be one of the essential differences between the methods employed. Group I was isolated without antibiotics. This group comprises solely patient specimens. Group II was isolated using penicillin + polymyxin B (media A, C and E, p. 62), and group III using penicillin + streptomycin + polymyxin B (medium B, p. 62).

Using this division, Table 19 (p. 74) shows the distribution of the 117 aerobic,

Table 19

Method of isolation	Number of strains	Indole production	Gelatine liquefaction	Nitrate reduction	Indole production, gelatin, liquefaction, no nitrate reduction	Antibiotic sensitivity identical to <i>Fl. mening.</i>
I. Isolated without use of antibiotics	20	13	14	0	10	5
II. Isolated using penicillin + polymyxin B (media A, C, E)	49	12	35	3	11	9
III. Isolated using penicillin + streptomycin + polymyxin B (medium B)	48	26	30	8	17	30
Totals	117	51	79	11	38	44

Some biochemical properties characteristic of *Flavobacterium meningosepticum* for 117 of the aerobic, oxidase-positive, non-motile strains shown in table 16 (pp. 64-71).

The strains have been divided in three groups I, II and III on the basis of the antibiotics used for the isolation (for the media used for the isolation see p. 62).

oxidase-positive non-motile strains with regard to some of the properties characteristic of *Flavobacterium meningosepticum*.

Indole production was found in 51 strains (44%), liquefaction of gelatine in 79 (68%), whilst nitrate reduction occurred in only 11 strains (9%). Only 5 of the 51 indole-positive strains reduced nitrate (groups 56-58 and 67, 68, Table 16), whilst 9 did not liquefy gelatine (groups 59-66 and 68). Table 19 also contains a column with those strains which possessed three of the cardinal properties of *Flavobacterium meningosepticum*, indole production, liquefaction of gelatine and lack of ability to reduce nitrate. 38 such strains were found.

Sensitivity pattern identical to that of Flavobacterium meningosepticum

A sensitivity pattern identical to that of *Flavobacterium meningosepticum* using Sensitabs (p. 42) is defined from the results given in Table 7 (p. 46) as follows: sulpa-methizole, penicillin and colimycin 0, tetracycline +, chloramphenicol, streptomycin and kanamycin +, +, +, erythromycin +, +, +, +.

Of the 117 strains shown in Table 19, 44 had this unusual sensitivity pattern (38%), while the pattern was found in 30 of the 48 (63%) strains isolated with penicillin + streptomycin + polymyxin B.

In Fig. 11 the ordinate shows the number of strains which showed the same degree of sensitivity as *Flavobacterium meningosepticum* to one or more of the eight

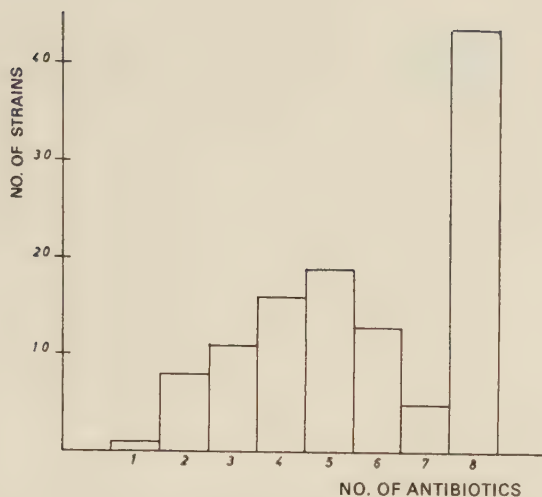


Fig. 11. Number of strains showing the same degree of sensitivity as *Flavobacterium meningosepticum* to one or more of the eight antibiotics studied. (the 117 strains shown in table 19 were investigated).

There is a sharp distinction between strains having the characteristic sensitivity pattern of *Flavobacterium meningosepticum* and the remainder, which seem to have a random distribution with regard to their sensitivity to the eight antibiotics.

antibiotics. Where the degree of sensitivity was the same for all eight antibiotics, this indicated that the sensitivity pattern was identical to that of *Flavobacterium meningosepticum*. As already mentioned, this was found in 44 out of the 117 strains. The figure shows that there is a sharp distinction between strains having the sensitivity pattern characteristic of *Flavobacterium meningosepticum* and the remainder, as only 5 strains had the same degree of sensitivity to seven antibiotics, whilst the remainder had even fewer. The strains in which the sensitivity pattern differed from *Flavobacterium meningosepticum* seemed to show a random distribution as regards degree of sensitivity to the eight antibiotics.

Acid production from carbohydrates

Table 20 (p. 76) shows the acid production by the 117 strains shown in Table 19 from the 21 carbohydrates which have been investigated. As in Table 19 the strains are divided into groups I, II and III according to the antibiotics which have been used in the isolation. The eight carbohydrates from which acid is produced by *Flavobacterium meningosepticum* are shown on the left of the table, and the 13 which it does not attack on the right.

Acid production from dulcitol, sorbitol and inositol was rare. The 15 strains which produced acid from adonitol all belonged to the group of 38 which did not liquefy gelatine. None of the 79 strains which liquefied gelatine produced acid from adonitol.

The 38 strains shown in Table 19 which possessed the three cardinal properties of

Table 20

Method of isolation	No. of strains	Glucose	Lævulose	Maltose	Lactose	Trehalose	Mannitol	Glycerol	Ethanol	Arabinose	Xylose	Rhamnose	Galactose	Sucrose	Raffinose	Starch	Inulin	Adonitol	Dulcitol	Sorbitol	Inositol	Salicin
I.....	20	17	11	15	6	8	5	13	7	6	7	2	7	4	2	8	0	3	0	1	0	3
II.....	49	29	28	26	12	19	5	21	12	18	19	10	16	14	10	12	7	6	2	2	1	7
III.....	48	46	36	42	19	43	13	35	19	23	23	12	12	27	15	16	13	6	3	4	3	13
Totals..	117	92	75	83	37	70	23	69	38	47	49	24	45	45	27	36	20	15	5	7	4	23

Acid production from 21 carbohydrates by 117 aerobic, oxidase-positive, non-motile strains.

The strains were divided into three groups I, II and III on the basis of the antibiotics used for the isolation, as in table 19. Strains in group I were isolated using no antibiotics, those in II using penicillin + polymyxin B and those in group III using penicillin + streptomycin + polymyxin B.

The eight carbohydrates attacked by *Flavobacterium meningosepticum* are shown to the left, the 13 not attacked to the right.

Flavobacterium meningosepticum (indole production, liquefaction of gelatine and lack of ability to reduce nitrate) differed from the latter mainly in that 15 of them produced acid from starch and 10 of them from sucrose, and also in that 24 of these strains did not attack mannitol.

The 23 strains which produced acid from salicin were all included among those strains which did not possess the three cardinal properties. This group also included all strains which produced acid from inulin, raffinose and rhamnose.

Combination of the properties indole formation, liquefaction of gelatine and lack of nitrate reduction, correlated to the carbohydrate pattern of Flavobacterium meningosepticum

In the following analysis*) the 117 aerobic, oxidase-positive, non-motile strains have been evaluated in order to determine whether the 38 strains which produced indole, liquefied gelatine and did not reduce nitrate, produced acid from the same carbohydrates as *Flavobacterium meningosepticum* more commonly than the remaining 79 strains.

As previously, the strains were divided into groups I, II and III according to the method used for isolation. Each of these groups was sub-divided according to whether or not the strains possessed the three cardinal properties of *Flavobacterium meningosepticum*. The strains in each sub-group are shown in Table 21 (p. 78) according to the number of carbohydrates out of the 21 tested to which they showed the same reaction as *Flavobacterium meningosepticum* as regards acid production. Weak carbohydrate reactions were considered to be deviating.

It may be seen from Table 21 that regardless of the method used in isolation those strains which possessed the three characteristic properties of *Flavobacterium meningosepticum* had the greatest number of reactions in common with the latter with regard to the production of acids from the same carbohydrates. The values for these strains in all three groups show a tendency to lie further to the right in the table than those for the strains which did not possess the three properties.

The 117 strains were in a similar manner divided according to the source from which they were isolated, rather than the method of isolation, in order to see whether the difference could be reproduced. This was found to be the case with the strains isolated from soil and from patient specimens, while very few strains with the three biochemical properties were isolated from river and tap water, and it was therefore not possible to demonstrate the difference for strains isolated from these sources.

The purpose of the method used in Tables 22 and 23 (p. 79) was to establish whether the difference just demonstrated applied equally to the eight carbohydrates attacked by *Flavobacterium meningosepticum* and the 13 which were not attacked by that bacterium.

Table 22 shows the 38 strains which produced indole, liquefied gelatine and did not reduce nitrate, whilst Table 23 shows the remainder. In both tables the column on the

*) The analysis has been made by the Biostatistical Department, Statens Seruminstitut.

Table 21

No. of carbohydrates with which the reaction was the same as Fl. mening.	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Total no. of strains
I. Indol-positive strains which liquefied gelatine and did not reduce nitrate.															
Remaining strains	0	0	0	0	0	0	1	2	3	1	1	0	2	0	10
Remaining strains	0	0	0	0	4	1	5	0	0	0	0	0	0	0	10
II. Indol-positive strains which liquefied gelatine and did not reduce nitrate.															
Remaining strains	0	0	0	0	0	0	3	1	3	1	2	0	1	0	11
Remaining strains	1	0	6	3	3	14	7	3	0	1	0	0	0	0	38
III. Indol-positive strains which liquefied gelatine and did not reduce nitrate.															
Remaining strains	0	0	0	0	0	0	0	1	1	8	3	4	0	0	18
Remaining strains	0	2	8	6	1	3	2	1	1	3	2	1	1	0	41
Total...															117

117 aerobic, oxidase-positive, non-motile strains. A total of 38 indol-positive strains which liquefied gelatine and did not reduce nitrate were compared with the remaining 79 with regard to whether they attacked the same carbohydrates as *Flavobacterium meningosepticum*. The 21 carbohydrates shown in table 20 were investigated. The strains were divided into three groups on the basis of the antibiotics used for the isolation. Each of the three groups was divided into two subgroups, one comprising the indol-positive strains which liquefied gelatine and did not reduce nitrate and the other all the remainder.

left shows the number of carbohydrates out of the eight attacked by *Flavobacterium meningosepticum* which were also attacked by the strains investigated, and the uppermost row, correspondingly, the number which were neither attacked by *Flavobacterium meningosepticum* nor by the strains concerned. The remaining figures indicate the number of strains which attacked and did not attack the number of carbohydrates given in the vertical and horizontal row, respectively.

In Table 22 the strains are found to accumulate within the framed rectangle in the

Table 22

Indol-positive strains which liquefied gelatine and did not reduce nitrate.

Common reactions to the 13 carbohydrates not attacked by Fl. mening.															Totals
Common reactions to the 8 carbohydrates attacked by F. mening.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	
0...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1...	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
2...	0	0	0	0	0	0	0	0	0	0	0	0	1	2	3
3...	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
4...	0	0	0	0	0	0	0	0	0	0	1	1	4	4	10
5...	0	0	0	0	0	0	0	0	0	0	0	0	3	1	4
6...	0	0	0	0	0	0	0	0	0	0	2	3	3	3	11
7...	0	0	0	0	0	0	0	0	0	1	0	2	1	3	7
8...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Totals....	0	0	0	0	0	0	0	0	0	1	3	6	13	15	38

Table 23

Remaining strains

Common reactions to the 13 carbohydrates not attacked by Fl. mening.															Totals
Common reactions to the 8 carbohydrates attacked by Fl. mening.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	
0...	0	0	0	0	0	0	0	0	0	0	0	0	0	13	13
1...	0	0	0	0	0	0	0	0	0	0	1	0	2	8	11
2...	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2
3...	0	0	0	0	0	0	0	0	1	0	1	0	0	1	3
4...	0	0	0	0	0	0	0	0	0	1	3	1	0	0	5
5...	0	0	0	0	2	7	3	1	0	2	2	0	1	0	18
6...	0	0	1	0	6	2	7	1	0	0	0	1	2	1	21
7...	0	0	0	1	2	0	0	0	0	0	1	0	0	1	5
8...	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Totals....	0	0	1	1	10	9	10	2	1	4	8	2	6	25	79

Tables 22 and 23 illustrate that the ability of indol-positive strains which liquefy gelatine the do not reduce nitrate to produce acid from the same carbohydrates as *Flavobacterium meningosepticum* (see table 21) depends mainly on the fact that they did not attack those carbohydrates which were not attacked by the latter.

lower right-hand corner, and in Table 23 there is a similar rectangle of about the same height, but a greater width. This implies that the demonstrated ability of the indole-positive strains which liquefied gelatine and did not reduce nitrate (Table 21) to attack the same carbohydrates as *Flavobacterium meningosepticum* is mainly due to a lack of ability to attack carbohydrates which were similarly not attacked by *Flavobacterium meningosepticum*.

The values contained within the framed areas in Tables 22 and 23 are distributed randomly, from which it may be concluded that there is no correlation between the capacity for attacking the eight and the 13 carbohydrates.

In addition to the areas already described there is a second area in the upper right-hand corner of Table 23 in which there is an accumulation of strains. This area indicates that 21 of the 79 strains which did not possess the three properties characteristic of *Flavobacterium meningosepticum* did not produce acid from any, or at most one, of the 21 carbohydrates. The majority of these strains were isolated from river water (groups 80-97, Table 16).

Isolation of Flavobacterium meningosepticum

On the basis of all the investigations, and the experience gained in this study, a description will now be given of the method which must be considered to be the most suitable for the isolation of *Flavobacterium meningosepticum*.

1. Inoculation on 5% horse blood agar plates containing 10 i.u. penicillin + 40 µg streptomycin + 40 µg polymyxin B per ml. Incubation at 35°C for 2 days.

2. Pure culture of the colonies on Conradi Drigalski plates. Incubation at 35°C for 2 days.

3. Inoculation into semi-solid agar and investigation for acid production from mannitol and salicin at 35°C (Hugh & Leifson's medium).

4. Aerobic, oxidase-positive, non-motile rods which produce acid from mannitol but not salicin should be investigated for indole production (p. 28), liquefaction of gelatine and reduction of nitrate.

When this technique was applied to 32 soil samples it was found that the 75 strains isolated differed only in minor respects from *Flavobacterium meningosepticum* as regards sensitivity to antibiotics and production of acid from carbohydrates (Table 18, p. 73).

The addition of 1% mannitol (medium D, p. 62) did not facilitate the isolation on the primary plates as the acid production was weak and difficult to recognize, regardless of whether bromthymol blue or phenol red was used as indicator.

DISCUSSION

The purpose of this part of the study was to isolate *Flavobacterium meningosepticum* outside the hospital, and it was found necessary to experiment with a number of

different media and methods of isolation (p. 62–64) before a suitable method was devised.

All the methods used were selective, as the media employed all contained antibiotics. The number of strains isolated cannot therefore be considered as an expression of the relative incidence of these strains in nature.

Strains which were identical to *Flavobacterium meningosepticum* as regards biochemical properties and sensitivity to antibiotics were isolated from river water, cultivated soil and specimens from patients, but the numbers isolated were small (Table 17, p. 72).

No antibiotics were used in the isolations from the specimens from patients, and the number of strains isolated may therefore be regarded as an expression of their incidence. Apart from those isolated from the patients with bacteraemia described in Chapter 3, during a period of 4 years investigation of 68,521 patient specimens yielded only 24 aerobic, oxidase-positive, non-motile strains, despite the fact that attention was paid particularly to these, and that it had regularly been possible to demonstrate *Flavobacterium meningosepticum* in the hospital surroundings. *Flavobacterium meningosepticum* is thus extremely rare in specimens from patients. It is interesting that strains identical to *Flavobacterium meningosepticum* were isolated from two specimens from patients in hospitals in Copenhagen (p. 26 and p. 72). There is a report from England of the isolation of 6 strains resembling *Flavobacterium meningosepticum* from specimens from patients (Greaves 1966). The micro-organism has not previously been isolated outside hospital

During this study the author gained the impression that there was a correlation between the properties indole production, liquefaction of gelatine and lack of reduction of nitrate, and the ability to produce acids from the same carbohydrates as *Flavobacterium meningosepticum*. It was possible to confirm this in a statistical evaluation of a material comprising 117 aerobic, oxidase-positive, non-motile strains. In this investigation it was necessary to exclude strains isolated by the selective method described on pp. 63–64, as only strains which produced indole, liquefied gelatine and did not reduce nitrate and which in addition produced acid from mannitol but not salicin, were included.

Summary

This study was started after the diagnosis of bacteraemia due to *Flavobacterium meningosepticum* in recently operated, adult patients in the Department of Thoracic Surgery, Odense County and City Hospital.

In regions with hot climates *Flavobacterium meningosepticum* has been responsible for 63 cases of severe meningitis in premature infants, but it has hitherto been found as a pathogen in adults in only one case.

The purpose of this study has been to describe the clinical picture in adult patients and to elucidate the epidemiology of *Flavobacterium meningosepticum*, to investigate its properties and its occurrence in nature.

CHAPTER 1

The serious prognosis of the meningitis in premature infants caused by *Flavobacterium meningosepticum* is described. It is mentioned that factors of importance in the antibiotic therapy of meningitis in the premature are that these infants have an increased meningeal permeability, and that the slow renal excretion means that the serum concentration following a dose of antibiotics is higher than the concentration following the corresponding dose in adults. Mention is also made of the fact that the effect of a number of antibiotics on various bacteria increases with increasing temperature, but that this is of no advantage in meningitis in premature infants, where there is often no fever.

CHAPTER 2

The importance of temperature for micro-organisms is discussed. It is shown (Table 1, p. 18) that obligate human pathogens can reproduce only in a narrow interval of temperature around 37°C, whilst bacteria which are pathogenic to man but also capable of living outside the human organism can grow over a wider range of temperature. Non-pathogenic bacteria generally have a temperature optimum other than 37°C. It is mentioned that there are many examples of mutants which deviate as regards thermosensitivity, and according to the results of the present study *Flavobacterium meningosepticum* would seem to be an example of these.

A brief summary is given of Arrhenius' law, which has been found to apply to a

number of biological processes, including, within a limited range of temperature, the growth of bacteria.

CHAPTER 3

A description is given of 11 adult patients from whom *Flavobacterium meningosepticum* was isolated after operation. One of the patients was from a hospital in Copenhagen. In contrast to the prognosis in *Flavobacterium meningosepticum* meningitis in premature infants, the prognosis in adult patients is good. Clinically the infection is characterized by a transient hyperthermia with little disturbance of the general condition and with no symptoms or signs from the central nervous system.

Agglutinating antibodies were demonstrated in the serum of eight out of nine patients.

CHAPTER 4

The biochemical properties of 13 strains of *Flavobacterium meningosepticum* were investigated. Characteristic properties were positive oxidase reaction, indole production, liquefaction of gelatine, and lack of ability to reduce nitrate. The micro-organism is a non-motile, gram-negative rod, which shows predominantly aerobic growth.

The bacterium was frequently isolated from basic environment, and growth was therefore investigated at various pH levels to determine whether a basic substrate could be used selectively for its isolation. However, *Flavobacterium meningosepticum* was found to be no more resistant to alkali than six other bacteria which were investigated by the same method (Table 3, p. 31).

Growth is facilitated by high oxygen tension to about the same extent as the growth of *Pseudomonas aeruginosa*, and to a greater extent than the growth of *Escherichia coli*. All three bacteria were capable of using all the free oxygen in a broth substrate. During growth in broth there was a fall in the oxidation-reduction potential of all three bacteria. The fall reached a minimum which coincided with the end of the phase of exponential growth (Figs. 3, 4 and 5, pp. 32–33).

CHAPTER 5

The clinical picture suggested that *Flavobacterium meningosepticum* was a thermolabile bacterium. The growth of 13 strains of *Flavobacterium meningosepticum* was therefore studied at a number of temperatures between 4° and 41°C.

An obvious difference was demonstrated between the strains isolated from adult patients with bacteraemia and those from premature infants with meningitis. The former already showed inhibition of growth at 37°C and almost no growth at 38°C (Tables 5 and 6, pp. 38–39). They were killed by incubation at 38°C for 4 days. The strains isolated from patients with meningitis did not show inhibition of growth until the temperature reached 40°C. They were killed by incubation at 41°C for 2 days.

These differences in thermoresistance may be the reason for the difference in

pathogenicity. The marked sensitivity to temperature in the bacteraemia strains provides an explanation for the finding that the adult patients with bacteraemia recovered spontaneously in connection with the development of fever, and it may also be the reason why these strains have not been isolated in regions with hot climates.

CHAPTER 6

The sensitivity of *Flavobacterium meningosepticum* to antibiotics has been investigated using an agar diffusion method and the tube dilution method. The micro-organism was found to be highly resistant to antibiotics. It showed greatest sensitivity to antibiotics which usually act on gram-positive bacteria. It was most sensitive to erythromycin, novobiocin and vancomycin, whilst there was extreme resistance to colimycin and sulphadimidine (Table 8, p. 47).

An investigation was made to see whether it was possible to achieve any synergistic action by combinations of antibiotics, but this was found to be so weak that it was not to be expected to be of any therapeutic interest.

CHAPTER 7

As *Flavobacterium meningosepticum* was highly resistant to antibiotics, but at the same time sensitive to temperature, an investigation was carried out to determine whether the sensitivity to antibiotics would be increased if the temperature were raised. This was found to be the case for erythromycin, novobiocin, fucidin, tetracycline, chloramphenicol, streptomycin and sulphadimidine, but not for penicillin, ampicillin or vancomycin (Table 13, p. 52).

CHAPTER 8

A review is given of the available means of treatment of *Flavobacterium meningosepticum* infections, based on the findings described in Chapters 5, 6 and 7. None of the antibiotics studied could be expected to give reliable results, and in meningitis it is always necessary to use intrathecal antibiotics. Penicillin in very high dosage is mentioned as one possible mode of treatment.

High temperatures would be an advantage, both from the viewpoint of the sensitivity of the bacterium to temperature and because of the greater effectivity of a number of antibiotics. In infections with thermolabile strains the rise in temperature will presumably be of crucial importance in fighting the infection, and hyperthermia therapy may be contemplated in such infections if the patient does not develop fever.

CHAPTER 9

A description is given of the epidemiological investigations which were carried out in connection with the hospital endemic described in Chapter 3. The source of in-

fection was contaminated multiple dose vials of drugs which were used for anaesthesia.

The micro-organism was found to be wide-spread throughout the hospital, particularly in such damp places as suction apparatus, outlet pipes, wash-basins and the air-conditioning apparatus. It was also demonstrated in the air of the operating theatres. No human carriers were demonstrated.

Despite the wide-spread occurrence in the environment only those patients who had received intravenous anaesthesia became ill. From this it is concluded that *Flavobacterium meningosepticum* is of low pathogenicity in man.

CHAPTER 10

An investigation was carried out to determine whether *Flavobacterium meningosepticum* or related strains could be demonstrated in soil, river water, tap water and specimens from patients in whom there was no clinical evidence of infection with *Flavobacterium meningosepticum*. Strains which were identical to *Flavobacterium meningosepticum* biochemically and in their sensitivity to antibiotics were demonstrated in small numbers in patient specimens and in soil and river water, but not in tap water (Table 17, p. 72).

Strains which differed only slightly from *Flavobacterium meningosepticum* were demonstrated in larger numbers (groups 3–55, Table 16, pp. 64–68).

The properties of all the aerobic, oxidase-positive, non-motile strains which were isolated are described. On statistical evaluation of these it was found that the properties indole production, liquefaction of gelatine and lack of ability to reduce nitrate, which are characteristic of *Flavobacterium meningosepticum*, were correlated to the carbohydrate pattern of that organism (Table 21, p. 78).

On the basis of all the investigations and the experience gained in this study a description is given of the method which is considered to be the most suitable for the isolation of *Flavobacterium meningosepticum* (p. 80). Using this method it was possible to isolate from 32 soil samples a total of 75 strains, none of which differed greatly from *Flavobacterium meningosepticum* (Table 18, p. 73).

Resumé

Arbejdet igangsattes efter diagnosticering af bakteriæmi med *Flavobacterium meningosepticum* hos nyopererede voksne patienter indlagt på Odense amts og bys sygehus, thoraxkirurgisk afdeling.

Flavobacterium meningosepticum har i områder med varmt klima i 63 tilfælde forårsaget svær meningitis hos præmature, men bortset fra ét tilfælde er den ikke tidligere fundet patogen hos voksne.

Arbejdets formål: Beskrivelse af det kliniske billede hos de voksne patienter og klarlæggelse af de epidemiologiske forhold. Undersøgelse af *Flavobacterium meningosepticum*s egenskaber og dens forekomst i naturen.

KAPITEL 1

Der omtales den alvorlige prognose ved præmatur meningitis forårsaget af *Flavobacterium meningosepticum*. Som værende af betydning for antibiotisk behandling af præmatur meningitis nævnes, at præmature har øget meningeal permabilitet, og at de ved samme antibiotikumdosering som voksne opnår en højere serumkoncentration på grund af langsommere renal udskillelse. Det omtales, at en række antibiotikas virkning på forskellige bakterier øges med temperaturen, hvad præmature med meningitis ikke kan drage fordel af, da de ofte ikke har feber.

KAPITEL 2

Temperaturens betydning for mikroorganismer diskuteres. Det anføres (tabel 1, p. 18), at obligat human patogene bakterier kun vokser i et snævert temperaturinterval omkring 37°C, medens human patogene bakterier, der tillige er i stand til at leve uden for den menneskelige organisme, vokser i et bredere temperaturinterval. Apatogene bakterier har i reglen et andet temperaturoptimum end 37°C. Det nævnes, at der findes flere eksempler på mutanter, der afviger med hensyn til temperaturfølsomhed, og efter egne undersøgelser er også *Flavobacterium meningosepticum* et eksempel på dette.

Kortfattet omtales Arrhenius lov, der er fundet gældende for en række biologiske processer og også inden for et begrænset temperaturinterval gælder for bakteriers vækst.

KAPITEL 3

Der omtales 11 voksne patienter fra hvilke *Flavobacterium meningosepticum* blev isoleret postoperativt. Én af patienterne var fra et københavnsk hospital. I modsætning til meningitis hos præmature forårsaget af *Flavobacterium meningosepticum* var prognosen hos de voksne patienter god. Klinisk var tilstanden karakteriseret af en udtalt, men kortvarig hypertermi med ringe påvirkning af almentilstanden og uden symptomer fra centralnervesystemet.

Agglutinerende antistoffer i serum påvistes hos otte af ni patienter.

KAPITEL 4

De biokemiske egenskaber undersøgtes for 13 *Flavobacterium meningosepticum* stammer. Karakteristiske egenskaber var oxidasedannelse, indoldannelse, gelatine-smeltning og manglende evne til at reducere nitrat. Mikroorganismen er en ubevægelig, gram-negativ stav, der overvejende vokser aerobt.

Bakterien isoleredes hyppigt fra basisk miljø, hvorfor væksten undersøgtes ved forskellige pH værdier med henblik på, om et basisk substrat kunne anvendes selektivt til isolering af bakterien. *Flavobacterium meningosepticum* fandtes dog ikke mere alkaliresistent end seks andre bakterier undersøgt med samme metode (tabel 3, p. 31).

Væksten fremmedes af høj ilttension i omtrent samme grad som væksten af *Pseudomonas aeruginosa* og i højere grad end væksten af *Eschericia coli*. Alle tre bakterier var i stand til at udnytte al fri ilt i et bouillonsubstrat. Under vækst i bouillon kom der for alle tre bakterier et fald i redoxpotential. Faldet nåede minimum samtidig med afslutningen af den eksponentielle vækstfase (fig. 3, 4 og 5, p. 32 og 33).

KAPITEL 5

Det kliniske billede gav formodning om, at *Flavobacterium meningosepticum* var en temperaturfølsom bakterie. Væksten af 13 *Flavobacterium meningosepticum* stammer undersøgtes derfor for en række temperaturer mellem 4° og 41°C.

Der påvistes en tydelig forskel mellem stammer isoleret fra de voksne patienter med bakteriæmi og stammer isoleret fra præmature med meningitis. De førstnævnte var væksthæmmet allerede ved 37°C og voksede næsten ikke ved 38°C (tabel 5 og 6, p. 38 og 39). De dræbtes efter fire døgn inkubation ved 38°C. Stammerne isoleret fra meningitis patienterne var først væksthæmmet ved 40°C. De dræbtes efter to døgn inkubation ved 41°C.

Disse forskelle i temperaturfølsomhed kan være årsag til forskellen i patogenitet. Den betydelige temperaturfølsomhed af bakteriæmistammen forklarer, at de voksne patienter med bakteriæmi helbredtes spontant i forbindelse med hypertermien, og kan også være en årsag til, at denne stamme ikke er isoleret i områder med varmt klima.

KAPITEL 6

*Flavobacterium meningosepticum*s følsomhed for antibiotika undersøgtes med en agardiffusinsmetode og med glasfortyndingsmetoden. Mikroorganismen fandtes meget resistent mod antibiotika. Den var mest følsom for antibiotika, der sædvanligvis virker på gram-positive bakterier. Størst følsomhed fandtes for erythromycin, novobiocin og vancomycin. Den var ekstrem resistent mod colimycin og sulfadimidin (tabel 8, p. 47).

Det undersøgtes, om en synergistisk virkning kunne opnås ved kombination af antibiotika, men dette fandtes i så ringe grad, at det ikke kan forventes at kunne få terapeutisk betydning.

KAPITEL 7

Da *Flavobacterium meningosepticum* var meget antibiotikaresistent, men samtidig ret temperaturfølsom, undersøgtes det om den antibiotiske følsomhed kunne øges, hvis temperaturen forhøjedes. Dette fandtes at være tilfældet for erythromycin, novobiocin, fucidin, tetracyclin, kloramfenicol, streptomycin og sulfadimidin, men ikke for penicillin, ampicillin og vancomycin (tabel 13, p. 52).

KAPITEL 8

På grundlag af resultaterne i kapitlerne 5, 6 og 7 gives en oversigt over behandlingsmulighederne ved *Flavobacterium meningosepticum* infektioner. Der vil ikke kunne forventes sikre resultater ved behandling med nogle af de undersøgte antibiotika, og ved meningitis må der altid doseres intraspinalt. Penicillinbehandling med store doser nævnes som en behandlingsmulighed.

Høj feber vil være gavnlig både på grund af bakteriens temperaturfølsomhed og på grund af en bedre virkning af en række antibiotika. Ved infektioner med den temperaturfølsomme stamme vil temperaturstigning formentlig være den afgørende faktor til bekæmpelse af infektionen, og hypertermibehandling kan ved sådanne infektioner overvejes, hvis patienten ikke har feber.

KAPITEL 9

Der omtales de epidemiologiske undersøgelser, der blev foretaget i forbindelse med den i kapitel 3 beskrevne hospitalsendemi. Infektionskilden var kontaminerede hætteglas med medikamenter, der anvendtes til anæstesi.

Mikroorganismen fandtes vidt udbredt på hospitalet især på fugtige steder som sug, afløb, vaske og luftfugteranlæg. Den påvistes også i luften på operationsstuer. Humane bærere påvistes ikke.

Trods den udbredte forekomst i omgivelserne blev kun patienter, der havde fået intravenøs anæstesi syge. Heraf konkluderes at *Flavobacterium meningosepticum* er lavpatogen for mennesker.

KAPITEL 10

Det undersøgtes om *Flavobacterium meningosepticum* og beslægtede stammer kunne påvises i jord, åvand, vandværksvand samt i prøver fra patienter, der ikke klinisk havde tegn på infektion med *Flavobacterium meningosepticum*. Stammer, der biokemisk og med hensyn til følsomhed for antibiotika var identiske med *Flavobacterium meningosepticum*, påvistes i et ringe antal fra patientprøver samt i jord og åvand, men ikke i vandværksvand (tabel 17, p. 72).

Stammer, der kun var lidt afvigende påvistes i et større antal (grupperne 3-55, tabel 16, p. 64-68)

Egenskaberne for samtlige isolerede aerobe, oxidasepositive, ubevægelige stave omtales. Ved en statistisk vurdering af disse påvistes det, at kombinationen af egenskaberne indoldannelse, gelatinesmeltning og manglende nitratreduktion, der er karakteristisk for *Flavobacterium meningosepticum*, var korreleret til *Flavobacterium meningosepticum*s kulhydratmønster (tabel 21, p. 78).

På grundlag af samtlige undersøgelser og indhøstede erfaringer beskrives den metode, der anses for den bedste til isolering af *Flavobacterium meningosepticum* (p. 80). Med denne metode isoleredes fra 32 jordprøver 75 stammer, der alle kun afveg lidt fra *Flavobacterium meningosepticum* (tabel 18, p. 73).

References

(The numbers in brackets refer to the pages in the monograph on which the publication is mentioned)

Textbooks

- Bourdillon, R. B., O. M. Lindwell, J. E. Lovelock & W. F. Raymond: Studies in Air Hygiene. Spec. Rep. Ser. med. Res. Coun. (Lond.) No. 262, 1948. (58).
- Breed, R. S., E. G. D. Murray & N. R. Smith: Bergey's Manual of Determinative Bacteriology, 7th ed., Baltimore 1957. (14, 19).
- Hewitt, L. F.: Oxidation-Reduction Potentials in Bacteriology and Biochemistry, Edinburgh 1950. (35).
- Johnson, F. H., H. Eyring & M. J. Polissar: The Kinetic Basis of Molecular Biology, N. Y. 1954. (22).
- Lamerpt, H.: Überwärmung als Heilmittel. Stuttgart 1948. (19).
- Precht, H., J. Christophersen & H. Hensel: Temperatur und Leben. Berlin 1955. (21).
- Wilson, G. S. & A. A. Miles: Topley and Wilson's Principles of Bacteriology and Immunity, 4th ed. vol. 1-2, Lond. 1955. (17,18).

Other references

- Alford, J.: Effect of incubation temperature on biochemical tests in the genera *Pseudomonas* and *Achromobacter*. *J. Bact.* 1960, 79: 591-594. (17).
- Arriagada, A.: Estado actual de las investigaciones sobre los efectos de 42° de temperatura sobre la virulencia del bacilo Koch y sobre la actividad de las substancias antibacterianas. *Rev. clin. esp.* 1952, 47: 17-19. (15).
- Audebaud, G., M. Ganzin, J. Ceccaldi & P. Merveille: Isolement d'un *Chromobacterium violaceum* à partir des lésions hépatiques observées chez un singe *Cercopithecus cephus*. *Ann. Inst. Pasteur* 1954, 87: 413-418. (19).
- Axline, S. G. & H. J. Simon: Clinical pharmacology of antimicrobials in premature infants. Kanamycin, streptomycin, and neomycin. *Antimicrobial Agents and Chemotherapy* 1964, 135-142. (15).
- Axline, S. G., S. J. Yaffe & H. J. Simon: Clinical pharmacology of antimicrobials in premature infants. Ampicillin, methicillin, oxacillin, neomycin, and colistin. *Pediatrics* 1967, 39: 97-108. (15).
- Barber, M.: Coagulase-positive staphylococci resistant to penicillin. *J. Path. Bact.* 1947, 59: 373-384. (45).
- Bartholomew, J. W. & S. C. Rittenberg: Thermophilic bacteria from deep ocean bottom cores. *J. Bact.* 1949, 57: 658-659. (20).
- Bazett, H. C.: Studies on the effects of baths on man. *Amer. J. Physiol.* 1924, 70: 412-451. (26).
- Berger, W.: Bisherige Erfahrungen mit einer kombinierten Chloromycetin-Sulfonamid-Pyrifer-Therapie bei typhösen Erkrankungen. *Dtsch. med. Wschr.* 1951, 76: 1592-1596. (16).
- Berry, B. W., A. G. Morrow, C. D. Harrison, H. D. Hochstein & C. K. Himmelsbach: Flavobacterium septicemia following intracardiac operations. *J. thorac. cardiovasc. Surg.* 1963, 45: 476-481. (14).

- Black, W. A., A. Pollock & E. L. Batchelor: Fatal transfusion reaction due to *Serratia marcescens*. *J. clin. Path.* 1967, 20: 883–887. (19).
- Black, M. E., J. Shanahan & F. Clearwater: *Bacillus violaceus* infection in a human being. *J. Amer. med. Ass.* 1938, 110: 1270–1272. (19).
- Boizot, G. E.: An examination of the modified Eijkman method applied to pure coliform cultures obtained from waters in Singapore. *J. Hyg. (Lond.)* 1941, 41: 566–570. (20).
- Bojsen-Møller, J.: Studies on the growth and isolation of *Listeria monocytogenes* at low temperatures. *Second Symposium on Listeric Infection, Montana State College*, 1962, p. 169–172. (19).
- Brock, T. D.: Micro-organism adapted to high temperatures. *Nature (Lond.)* 1967, 214: 882–886. (20).
- Brody, A. J., H. Moore & E. O. King: Meningitis caused by an unclassified gram-negative bacterium in newborn infants. *Amer. J. Dis. Child.* 1958, 96: 1–5. (13, 61).
- Buttiaux, B. & J. Vandepitte: Les *Flavobacterium* dans méningites épidémiques des nouveau-nés. *Ann. Inst. Pasteur* 1960, 98: 398–404. (13, 40).
- Cabrere, A. H. & G. H. Davis: Epidemic meningitis of the newborn caused by *Flavobacteria*. *Amer. J. Dis. Child.* 1961, 101: 289–295. (13, 27, 61).
- Cannefax, G. R.: A temperature-gradient bar and its applications to the study of temperature effects on the growth of Reiter's treponeme. *J. Bact.* 1962, 83: 798–710. (17).
- Chen, K. K., R. C. Anderson, F. G. Henderson & C. A. Mills: Potency of cymarin and coumagine hydrochloride as influenced by environmental temperature. *Proc. Soc. exp. Biol. (N.Y.)* 1943, 53: 200–203. (16).
- Chen, K. K., R. C. Anderson, F. A. Steldt & C. A. Mills: Environmental temperature and drug action in mice. *J. Pharmacol. exp. Ther.* 1943, 79: 127–133. (16).
- Christensen, W. B.: Urea decomposition as means of differentiating *Proteus* and *Paracolon* cultures from each other and from *Salmonella* and *Shigella* types. *J. Bact.* 1946, 52: 461–466. (28).
- Christoffersen, K. J. & V. Hove: Temperatures indflydelse på virkningen af kombinationer af antibiotica in vitro. *Ugeskr. Læg.* 1967, 129: 885–891. (15).
- Cousin, D.: Mutants thermosensibles d'*Escherichia coli* KI. 2. *Ann. Inst. Pasteur* 1967, 113: 309–326. (19).
- Crozier, W. J.: On the critical thermal increment for locomotion of a diplopod. *J. gen. Physiol.* 1925, 7: 123–137. (22).
- Davidson, C. M., M. Boothroyd & D. L. Georgala: Thermal resistance of *Salmonella senftenberg*. *Nature (Lond.)* 1966, 212: 1060–1060. (19).
- Dernby, K. G.: La concentration optima en ions hydrogène favorisant le développement de certains micro-organismes. *Ann. Inst. Pasteur* 1921, 35: 277–291. (34).
- Dernby, K. G. & C. Näslund: Die Beziehung der Wachstumskurven einiger Mikroorganismen der Dysenterie-Coli-Gruppe zur Wasserstoffionkonzentration. *Z. Immun.-Forsch.* 1923, 35: 450–455. (34).
- Dewhurst, K. & J. Todd: The effect of fever on the penetration of penicillin into the cerebrospinal fluid. *Int. J. Neuropsychiat.* 1965, 257–263. (15).
- Dobell, A. R. C., J. D. Wyant, K. B. Seamans & P. Gloor: Penicillin epilepsy. *J. thorac. cardiovasc. Surg.* 1966, 52: 469–475. (56).
- Dons, N.: Eksperimentelle og kliniske undersøgelser over effekten af penicillin over for infektioner med penicillinasedannende stafylokokker. Thesis, Copenhagen 1966, p. 52–54. (47).
- Dorn, F. L. & R. Rahn: Definition versus measurement of optimal temperature. *Arch. Microbiol.* 1939, 10: 6–13. (17).
- Drost-Hansen, W.: Temperature anomalies and biological temperature optima in the process of evolution. *Naturwissenschaften* 1956, 43: 512. (22).
- Eriksen, K. R. & S. Jarnum: Generaliseret infektion med *Serratia marcescens*. *Ugeskr. Læg.* 1960, 122: 442–444. (19).

- Eriksen, K. R. & I. Erichsen: Resistance to methicillin, isoxazolyl penicillins and cephalothin in staphylococcus. *Acta path. microbiol. scand.* 1964, 62: 255–275. (45).
- Fassin, W., R. Hengel & P. Klein: Bakteriostase und Bakterizidie als Alternativen des antibakteriellen Chloramphenicoleffektes. *Z. Hyg. Infekt.-Kr.* 1955, 141: 363–375. (15).
- Feigen, G. A., N. S. Peterson, W. W. Hofmann, G. H. Genthner & W. E. van Heyningen: The effect of impure tetanus toxin on the frequency of miniature end-plate potentials. *J. gen. Microbiol.* 1963, 33: 489–495. (22).
- Ferramola, R.: Wilson's method for the bacteriological examination of water. *Amer. J. publ. Hlth* 1940, 30: 1083–1091. (20).
- Gale, E. F. & H. M. R. Epps: The effect of the pH of the medium during growth on the enzymic activities of bacteria (*Escherichia coli* and *Micrococcus lysodeikticus*) and the biological significance of the changes produced. *Biochem. J.* 1942, 36: 600–617. (34).
- Garrod, L. P.: The penicillins. In: *Experimental Chemotherapy* (Eds. R. J. Schnitzer & F. Hawking), N.Y. & Lond. 1964, 3: 10. (56).
- Garrod, L. P. & P. M. Waterworth: Methods of testing combined antibiotic bactericidal action and the significance of the results. *J. clin. Path.* 1962, 15: 328–339. (43).
- George, R. M., C. F. Cochran & W. E. Wheeler: Epidemic meningitis of the newborn caused by *Flavobacteria*. *Amer. J. Dis. Child.* 1961, 101: 296–305. (13, 55).
- Gluck, L. & W. A. Silverman: Phagocytosis in premature infants. *Pediatrics* 1957, 20: 951–958. (15).
- Gluck, L., F. W. Harrison & D. F. Milfred: Septicemia of the newborn. *Pediat. Clin. N. Amer.* 1966, 13: 1131–1149. (15).
- Godtfredsen, W. O.: *Fusic acid and some related antibiotics*. Thesis, Copenhagen 1967, p. 65–66. (54).
- Graevenitz, A.: Über *Serratia*-Infektionen beim Menschen. *Path. et Microbiol. (Basel)* 1964, 27: 235–247. (19).
- Greaves, P. W.: Isolation of bacteria resembling *Flavobacterium meningosepticum* from human material in Britain. *J. med. Lab. Technol.* 1966, 23: 115–118. (81).
- Greene, R.: The effect of temperature upon nitrogen fixation by *Azotobacter*. *Soil Science* 1932, 33: 153–162. (20).
- Groover, R. V., J. M. Sutherland & B. H. Landing: Purulent meningitis of newborn infants. *New Engl. J. Med.* 1961, 264: 1115–1122. (15).
- Hahn, F. E.: Substances of microbial origin as inhibitors of protein synthesis. *IX Int. Congr. Microbiol.* Moscow July 1966. (54).
- Hamon, Y. & Y. Peron: Thermosensibilité d'une souche de *Proteus vulgaris*. *Ann. Inst. Pasteur* 1967, 112: 291–294. (19).
- Haxthausen, H.: *Kortfattet lærebog i hud og kønssygdomme*. Munksgaard, Copenhagen 1949, p. 248–250. (18).
- Hill, L. R.: An index to desoxyribonucleic acid base compositions of bacterial species. *J. gen. Microbiol.* 1966, 44: 419–437. (14).
- Hoagland, H.: Pacemakers of human brain waves in normals and general paretics. *Amer. J. Physiol.* 1936, 116: 604–616. (22).
- Hoagland, H. & C. T. Perkins: Some temperature characteristics in man. *J. gen. Physiol.* 1934–35, 18: 399–409. (22).
- Hugh, R. & E. Leifson: The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. *J. Bact.* 1953, 66: 24–27. (28, 80).
- Ingraham, J. L.: Temperature relationships. In: *The bacteria* (Eds. I. C. Gunsalus & R. Y. Stanier) Acad. Press, N.Y. 1962, 4: 265–296. (22).
- Jacobowsky, B.: General paresis and civilization. *Acta psykiat. scand.* 1965, 41: 267–274. (19).
- Jennison, M. W.: Some quantitative relationships in bacterial population cycles. *J. Bact.* 1935, 30: 603–624. (19).

- Jensen, K. A.: *Almen kemi*. Gjellerup, Copenhagen 1959, 2: 357–360. (21).
- Jessen, O.: *Pseudomonas aeruginosa and other green fluorescent pseudomonads*. Thesis, Munksgaard, Copenhagen 1965. (19, 30).
- Johnson, F. H.: In: *Microbial ecology* (Eds. R. E. O. Williams & C. C. Spicer) 7th Symp. Soc. gen. Microbiol. Cambridge 1957, p. 145–153. (22).
- Jøker, R. N., T. Nørholm & K. E. Siboni: A case of neonatal meningitis caused by a yellow Enterobacter. *Dan. med. Bull.* 1965, 12: 128–130. (31).
- King, E. O.: Studies on a group of previously unclassified bacteria associated with meningitis in infants. *Amer. J. clin. Path.* 1959, 31: 241–247. (13, 28, 40, 48, 57, 60).
- King, E. O.: Personal communication 1965. (13, 27, 60).
- Knox, R., M. B. King & R. C. Woodroffe: In-vitro action of isoniazid on Mycobacterium tuberculosis. *Lancet* 1952, 2: 854–859. (15).
- Kovacs, N.: Identification of Pseudomonas pyocyanea by the oxidase reaction. *Nature (Lond.)* 1956, 178: 703. (28).
- Langenskiöld, F.: *Nordisk lærebok i kirugi*. Munksgaard, Copenhagen 1949, 3: 369–371. (18).
- Laporte, A., D. Fritel, G. Ricordeau & C. Betourne: Le problème des rechutes dans les fièvres typhoïdes et paratyphoïdes traitées par la chloromycétine. *Presse méd.* 1950, 58: 989–992. (16).
- Lautrop, H.: Gelatin-liquefying Klebsiella strains. *Acta path. microbiol. scand.* 1956, 39: 375–384. (28).
- Lavergne, M. & W. Drost-Hansen: Discontinuities in slope of the temperature dependence of the thermal expansion of water. *Naturwissenschaften* 1956, 43: 511–512. (22).
- Lefèvre, J.: *Chaleur animale et bioénergétique*. Paris 1911, p. 332–333. (17).
- Leifson, E.: Indole production by bacteria. *Int. J. syst. Bact.* 1966, 16: 171–172. (34).
- Lerner, P. I., H. Smith & L. Weinstein: Penicillin neurotoxicity. *Ann. N.Y. Acad. Sci.* 1967, 145: 310–318. (56).
- Loewy, A. & H. Gerhartz: Über die Temperatur der Expirationsluft und der Lungenluft. *Arch. ges. Physiol.* 1914, 155: 231–245.
- Longmuir, I. S.: Respiration rate of bacteria as a function of oxygen concentration. *Biochem. J.* 1953, 57: 81–88. (34).
- Lyon, D. M.: The relation of pulse-rate to temperature in febrile conditions. *Quart. J. Med.* 1927, 20: 205–211. (26).
- Maas, W. K. & B. D. Davis: Production of an altered pantothenate-synthesizing enzyme by a temperature-sensitive mutant of Escherichia coli. *Proc. nat. Acad. Sci. (Wash.)* 1952, 38: 785–798. (19, 21).
- Mackie, T. J. & J. E. McCartney: *Handbook of bacteriology*. 10th Ed. Edinburgh & Lond. 1962, p. 355. (36, 43).
- Marks, J.: Effects of hyperthermia and antibacterial agents on tubercle bacilli. *Brit. med. J.* 1951, 2: 1318–1321. (15).
- McBee, R. H. & V. H. McBee: The incidence of thermophilic bacteria in arctic soils and waters. *J. Bact.* 1956, 71: 182–188. (20).
- Moffet, H. L. & T. Williams: Bacteria recovered from distilled water and inhalation therapy equipment. *Amer. J. Dis. Child.* 1967, 114: 7–12. (72).
- Møller, V.: Diagnostic use of the Braun KCN test within the Enterobacteriaceae. *Acta path. microbiol. scand.* 1954, 34: 115–126. (28).
- Møller, V.: Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. *Acta path. microbiol. scand.* 1955, 36: 158–172. (28).
- Nakae, T.: Method of temperature-gradient incubation and its application to microbiological examinations. *J. Bact.* 1966, 91: 1730–1736. (17, 18).
- Olsen, H.: The importance of temperature for the growth of Flavobacterium meningosepticum. *Acta path. microbiol. scand.* 1966, 67: 291–302. (39, 40, 556).

- Olsen, H.: A clinical analysis of 10 cases of postoperative infection with *Flavobacterium meningosepticum*. *Dan. med. Bull.* 1967a, 14: 1–5. (26).
- Olsen, H.: An epidemiological study of hospital infection with *Flavobacterium meningosepticum*. *Dan. med. Bull.* 1967b, 14: 6–9. (57).
- Olsen, H.: An in vitro study of the antibiotic sensitivity of *Flavobacterium meningosepticum*. *Acta path. microbiol. scand.* 1967c, 70: 601–612. (45, 47).
- Olsen, H., W. C. Frederiksen & K. E. Siboni: *Flavobacterium meningosepticum* in 8 non-fatal cases of postoperative bacteraemia. *Lancet* 1965, 1: 1294–1296. (27).
- Oppenheimer, C. H. & W. Drost-Hansen: A relationship between multiple temperature optima for biological systems and the properties of water. *J. Bact.* 1960, 80: 21–24. (17, 22).
- Otila, E.: Studies on the cerebrospinal fluid in premature infants. *Acta paediat. (Uppsala)* 1948, 35: Suppl. 8. (15, 56).
- Owen, T. R. & C. E. Hill: The effect of increased oxygen partial pressures on the growth of *Pseudomonas aeruginosa* in a closed aeration system. *J. gen. Microbiol.* 1965, 41: ii. (33).
- Parada, J. L. & M. V. Ortega: Growth inhibition by hexoses of a temperature sensitive thiazoles mutant of *Salmonella typhimurium*. *J. Bact.* 1967, 94: 707–712. (19, 21).
- Perret, C. J.: Idometric assay of penicillinase. *Nature (Lond.)* 1954, 174: 1012–1014. (44).
- Peterson, A. C., J. J. Black & M. F. Gunderson: Staphylococci in competition. *Appl. Microbiol.* 1964, 12: 70–76. (34).
- Pinter, M. & J. Ivanyi: Septicaemia due to *Flavobacterium* and *Mima* polymorpha. *Brit. med. J.* 1965, 1: 1555. (13).
- Plotkin, S. A. & J. C. McKittrick: Nosocomial meningitis of the newborn caused by a *Flavobacterium*. *J. Amer. med. Ass.* 1966, 198: 662–665. (13, 48, 55, 61).
- Raghavachari, T. N. S. & P. V. S. Iyer: The coli aerogenes index of pollution used in the bacteriological analysis of water. *Ind. J. med. Res.* 1939, 26: 867–875. (20).
- Richards, R. K.: Influence of fever upon action of 3,3-methylene-bis-(4ihydroxycoumarin) (Dicumarol). *Science* 1943, 97: 313–314. (16).
- Schiff, J., L. S. Suter, R. D. Gourley & W. D. Sutliff: *Flavobacterium* infection as a cause of bacterial endocarditis. *Ann. intern. Med.* 1961, 55: 499–506. (14).
- Seligmann, S. J.: Methicillin-resistant staphylococci: Genetics of the minority population. *J. gen. Microbiol.* 1966, 42: 315–322. (45).
- Seligman, R., M. Komarov & R. Reitler: *Flavobacterium meningosepticum* in Israel. *Brit. med. J.* 1963, 2: 1528–1529. (13, 61).
- Shulman, H. B. & M. S. Johnson: A case of meningitis in a premature infant due to a proteolytic bacillus. *J. Lab. clin. Med.* 1944, 29: 500–507. (13).
- Siboni, K. E.: *Staphylococcal endemia and prophylaxis*. Thesis, Munksgaard, Copenhagen 1960. (59).
- Stenderup, A., Færgeman, O. & M. Ingerslev: *Serratia marcescens* in premature infants. *Acta path. microbiol. scand.* 1966, 68: 157–160. (19).
- Sugathadasa, A. A. & S. N. Arseculeratne: Neonatal meningitis caused by a new serotype of *Flavobacterium meningosepticum*. *Brit. med. J.* 1963, 1: 37–38. (13, 40, 49).
- Tanner, J. M.: The relationships between the frequency of the heart, oral temperature and rectal temperature in man at rest. *J. Physiol.* 1951, 115: 391–409. (26).
- Thomsen, V. F.: *Om teknikken ved resistensbestemmelse med særligt henblik på anvendelse af prædiffusion*. Thesis, Copenhagen 1967. (42, 49).
- Timoneeff-Ressovsky, N. W.: Über geographische Temperaturreassen bei *Drosophila funebris* F. *Arch. Naturgesch.* 1935, N.F. 4: 245–258. (20).
- Vandepitte, J., Ch. Beeckmans & R. Buttiaux: Quatre cas de méningite à *Flavobacterium* chez des nouveau-nés. *Ann. Soc. belge Med. trop.* 1958, 38: 563–570. (13, 48, 61).
- Vandepitte, J., R. Eeckels & V. Seynhaeve: Infections neo-natales à *Flavobacterium meningosepticum*. *Ann. Soc. belge Med. trop.* 1965, 45: 52–72. (13, 49).

- Waterman, N. G., F. S. Danziger & B. L. Abrams: The effect of temperature on the sensitivity of *Staphylococcus aureus* to different antibiotics. *Surg. Gynec. Obstet.* 1967, p. 262-269. (15).
- Watson, K. G., J. G. Krogh & D. T. Jones: Neonatal meningitis caused by *Flavobacterium meningosepticum* type F. *J. clin. Path.* 1966, 19: 79-81. (13, 49, 61).
- Weinstein, L., M. Goldfield & D. Adamis: A study of intrathecal chemotherapy in bacterial meningitis. *Med. Clin. N. Amer.* 1953, 37: 1363-1378. (56).
- White, H. J.: The relationship between temperature and the streptococcal activity of sulphanilamide and sulphapyridine in vitro. *J. Bact.* 1939, 38: 549-562. (15).
- Wilson, G. S., R. S. Twigg, R. C. Wright, C. B. Hendry, M. P. Cowell & I. Maier: The bacteriological grading of milk. *Spec. Rep. Ser. med. Res. Coun. (Lond.)* No. 206, 1935. (20).
- Wodzinski, R. J. & W. C. Frazier: Moisture requirements of bacteria. *J. Bact.* 1960, 79: 572-578. 34.
- ZoBell, C. E.: Bacterial life at the bottom of the Philippine trench. *Science* 1952, 115: 507-509. (20).

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